

AN EVOLUTIONARY ANALYSIS OF CYTOCHROME c_6 AT THE STRUCTURAL AND FUNCTIONAL LEVEL

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It is a well-established fact that plastocyanin (Pc) is the only carrier of electrons from cytochrome b_6/f to photosystem I (PSI) in higher plants, although it can be replaced by cytochrome c_6 (Cyt) in many cyanobacteria and eukaryotic algae. The synthesis of one or another protein is regulated by the content in copper of the growth medium [1].

In the last years, we have performed a comparative analysis of the structural and functional features of Cyt and Pc in order to understand how the two metalloproteins are able to play the same physiological role despite their different structures. On the surfaces of the two proteins, a striking homology can be found between the arrangements not only of charged but hydrophobic residues. In fact, each protein possesses two equivalent interaction areas, one hydrophobic for electron transfer (site 1) and the other electrostatic for complex formation (site 2), which are similarly used for the interaction with both cytochrome b_6/f and PSI. At site 1, the redox pathway for electrons going into and out the respective metal atom is formed by the surface accessible ring C of the heme group in Cyt and copper-ligand His87 in Pc. Our site-directed mutagenesis study of cyanobacterial Cyt and Pc indeed revealed the existence of a single arginyl residue in their respective amino acid sequences that is similarly located between sites 1 and 2 of each protein and appears to play the same crucial role in the electron transfer to PSI [see ref. 2 for a recent review].

Cyt and Pc are thus an excellent case study of convergent evolution. A wide range of photosynthetic organisms (cyanobacteria, green algae and plants) were used to investigate the evolutionary pathway of the reaction mechanism of PSI reduction by these two proteins. From such an evolutionary analysis, a hierarchy of kinetic models with a significant increase in efficiency was proposed. PSI reduction by the donor proteins Cyt and Pc, isolated from different sources, could thus follow either an oriented collisional mechanism (type I), a mechanism requiring complex formation (type II), or complex formation with rearrangement of the interface (type III). This was interpreted by assuming that the reaction mechanism of each protein in the whole range of photosynthetic organisms has evolved from type I to type II and III up to reach the maximum efficiency [3].

Within this context, we proposed that Cyt was first “discovered” by Nature, when iron was much more available than copper because of the reducing

character of the primitive Earth's atmosphere. The relative bioavailabilities of iron and copper were going down and up, respectively, as long as the metals were being oxidized because of the increasing atmospheric dioxygen content [4]. In consequence, Cyt could have been replaced with Pc in cyanobacteria and eukaryotic algae and completely eliminated in plants, where the copper protein became a constitutively synthesized protein.

The absence of Cyt in plants was a widely accepted paradigm for many years until a number of plant genomes became sequenced. In 2002, a modified Cyt was discovered in some plants and it was proposed that such a cytochrome could replace Pc in *Arabidopsis* [5,6]. However, our structural and functional analysis of plant Cyt compared with plant Pc and algal Cyt allowed us to conclude that the plant heme protein is not an effective donor to its own PSI. The physicochemical parameters and surface electrostatic potential of Cyt and Pc from plants are so different that the latter could not be replaced by the former, and so the true function of plant Cyt may be related to an extra loop of 12 residues that is a unique feature of the plant heme protein [7]. *In vivo* experiments with plants mutated in both of the two Pc-coding genes also demonstrate that only Pc can donate electrons to PSI in plants [8].

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