Hypothesis

DOES THE CHLOROPLAST CONTROL MITOCHONDRIAL FUNCTIONS?

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Mitochondria and chloroplasts are semi-autonomous organelles. Their DNA code for only a part of their proteins so that they import nuclear-coded proteins which are synthetized on the 80 S ribosomes of the cytosol. While this aspect is well documented [1] much less is known of the possible interdependence of the 2 organelles in a plant cell and of the possible export of organelle proteins.

In the unicellular green alga, *Chlamydomonas reinhardtii* chloroplast protein synthesis appears to be essential for growth even under heterotrophic conditions when photosynthesis is not essential for growth. The *eryM2* mutants which have impaired chloroplast 70 S ribosomes display a cryo-sensitive growth under both phototrophic and heterotrophic conditions [2,3]. The *ac20* mutant of *C. reinhardtii* is deficient in assembly of chloroplast 70 S ribosomes [4,5]. We observed that this mutant also shows cryo-sensitive growth under both phototrophic and heterotrophic conditions (unpublished). In *Euglena*, bleached mutants were first thought to have lost their chloroplast DNA. In fact, they contain a defective chloroplast genome present at a low copy number in which the ribosomal RNA genes are preferentially retained [6,7]. This suggests that bleached mutants may have retained their chloroplast translational machinery.

Such large deletions of chloroplast genome are not known in *Chlamydomonas*. However, the colorless related alga *Polytoma obtusum* has also retained specific translational machinery in its leucoplast whose DNA very likely consists of highly repetitive simple sequences. A high degree of homology was found between the chloroplast rRNA of *C. reinhardtii* and the leucoplast rRNA of *P. obtusum* as well as between the cytoplasmic rRNA of both organisms [8–10].

Thus, if chloroplast protein synthesis is indispensable for cellular growth even under heterotrophic conditions, the question is raised of the nature of the chloroplast products that are required for cell survival. In *C. reinhardtii*, it was postulated that a chloroplast gene product synthesized on chloroplast ribosomes was required for nDNA replication [11]. In *Nicotiana tabacum*, a plastome mutant displaying maternal inheritance exhibited both chloroplast and mitochondrial defects [12]. In *N. tabacum*, a mutant was isolated displaying maternal inheritance, with altered chloroplasts and mitochondria [13]. This chloroplast mutant is resistant to streptomycin and has impaired chloroplast 70 S ribosomes [14]. When grown in the presence of streptomycin, the fine structure of the mitochondria remains intact in this mutant whereas that of the wild-type is altered. It was shown that streptomycin did enter the cell in the mutant as well as in the wild-type. Similarly growth of *C. reinhardtii* and *E. gracilis*, in the presence of chloramphenicol, induces ultrastructural changes of mitochondria of the same nature as those observed in non-photosynthetic organisms [15,16]. In *C. reinhardtii*, a survey [17] of chloroplast genes affecting organelle ribosomes led to the postulate that a mutation in a single chloroplast gene may result in alteration of both chloroplast and mitochondrial ribosomes [17]. This conclusion derives from the properties of the chloroplast mutants which possess chloroplast ribosomes resistant in vitro to 70 S translation inhibitors. These mutants can grow photosynthetically in the presence of a given antibiotic but also heterotrophically in the presence of the same antibiotics when respiring acetate in the dark. We reported a similar observation with a chloroplast mutant resistant to chloramphenicol [18]. We also observed that 2 chloroplast mutants which are streptomycin-dependent for phototrophic growth are also dependent on the same antibiotics for heterotrophic growth (unpublished). Up to now no mutant with maternal heredity could be isolated

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which is resistant to a 70 S translation inhibitor under phototrophic growth but sensitive under heterotrophic growth.

We reinvestigated nuclear mutants affecting chloroplast ribosomes in *C. reinhardtii*. The *eryM1* mutants are located in the structural gene of the *LC6* protein of chloroplast ribosomes [19]. We observed that these mutants also show resistance to erythromycin both under phototrophic and heterotrophic growth. In crosses, among >50 tetrads we observed a strict co-segregation of the resistance under both growth conditions. As we pointed out, we observed that the assembly mutant *ac20* shows cryo-sensitive growth both under phototrophic and heterotrophic conditions and we failed to separate both characters by crosses. The *eryM2* mutant which shows altered erythromycin-binding capacity, also shows cryo-sensitive growth under both phototrophic and heterotrophic conditions.

Thus, mutants with altered chloroplast 70 S ribosomes, whether of nuclear or chloroplast origin, are affected not only under phototrophic growth conditions but also when respiring acetate in the dark.

Taken together, these observations favour the conclusion that at least one protein of chloroplast origin is required for mitochondrial function which would consequently account for the non-dispensible character of chloroplast protein synthesis. If mitochondrial protein synthesis is resistant to 70 S translation inhibitors as the chloroplast is, one should further consider that an important homology exists between mitochondrial and chloroplast ribosomes. Some proteins common to both organelle ribosomes would be encoded by the nucleus whereas some would be encoded by the chloroplast. (About 25% of the chloroplast ribosomal proteins are of chloroplast origin in spinach, Mache, personal communication.) The continuity of the external membranes of chloroplast and mitochondria which was demonstrated in *Pteris vittata* and *Spinacia oleracea* may be relevant to the process of transfer of protein from the chloroplast to the mitochondria [20,21].

As eukaryotic cells are thought to require an intact mitochondrial compartment even when respiration is dispensable [22], one should further question whether plant cells require a chloroplast compartment to perform functions different from photosynthesis or protein synthesis.

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