A DNase-sensitive twisted structure in the mitochondrial matrix of *Polysiphonia* (Rhodophyta)

G. Tripodi, P. Pizzolongo, and M. Giannattasio. From the Istituto di Botanica Generale, Facoltà di Agraria, I-80055 Portici, Italy

In the course of observations concerning ultrastructural alterations in cellular organelles during the life cycle of red algae, our attention was attracted by a feature shown by mitochondria during the cell division which gives rise to young carpospores in *Polysiphonia*. In this note we will describe an interesting twisted structure in the mitochondrial matrix, which seems to differ from the helices seen in the intracisternal space by several other authors (2, 4, 6, 8, 9, 10).

**Materials and Methods**

Observations were carried out on Karnovsky-osmium fixed cystocarps of *Polysiphonia sertularioides* (Grat.) J.Ag. embedded in Araldite (7, 11). Ultrathin sections were stained with uranyl acetate and lead citrate, and observed with a Philips EM 300 electron microscope. Adjacent sections were floated on 0.2 M acetate buffer, pH 5.8, containing DNase (1.5 mg/ml), after osmium removal with H₂O₂. The digestion was carried out for 4 hr at 37°C. DNase, RNase-free and electrophoretically purified, was supplied from Sigma Chemical Co., St. Louis Mo. Control sections were floated on the buffer alone. Extracted and control sections were then stained as described above.

**Results and Discussion**

Mitochondria occurred in high frequency in the cells studied and showed an atypical organization of cristae very different from the one observed in other stages. The electron-transparent area of the mitochondrial matrix showed either DNA fibrils or a twisted structure (Figs. 1–5). Sometimes both inclusions were detected together and appeared to be intimately associated (Fig. 4). The twisted structure suggests a circular configuration of two coiled threads and shows a periodicity of about 200 A. This structure was observed in about 10% of the mitochondrial profiles, while the electron-transparent area of the matrix was lacking of both coils and fibrils in about 37% of the observed mitochondria. These percentages were computed on about 1000 mitochondrial profiles.

After DNase treatment on floating sections (Fig. 6), neither fibrillar structures nor twisted inclusions were recognizable in all the observed mitochondria (about 150). These structures were observed in the control sections floated on buffer alone. Fibrillar structures in plastids known to be DNA (3) were also removed by the above treatment.

A number of spiral mitochondrial inclusions have been described by Mugnaini (8), Svoboda and Higginson (10), Behnke (2), Jessen (6), Schuster (9), and Blecher (4). All of these spirals are rather similar morphologically and are localized within the intracisternal space; they sometimes appear to be arranged in parallel arrays and show variable periodicity between 120 A (Mugnaini) and 360 A (Blecher). These variations have been interpreted as probably due to the different states of the mitochondrial activity cycle (4). A correct interpretation of these spirals is quite a problem. Those observed by the authors mentioned have...
FIGURES 1, 2, and 3  The twisted threads in the mitochondrial matrix: the structure suggests a circular configuration. Fig. 1, $\times$ 60,000; Fig. 2, $\times$ 48,000; Fig. 3, $\times$ 40,000.

FIGURE 4  DNA fibrils showing continuity with the twisted structure. $\times$ 48,000.

FIGURE 5  Same as Figs. 1-3. $\times$ 54,000.

FIGURE 6  The mitochondrial matrix after DNase digestion. $\times$ 42,000.
been interpreted as possibly protein (8) or phospholipid in nature (10), although Blecher (4) suggests, on the basis of DNase digestions, that they contain DNA.

The helical structures we have observed are different from those previously described in their localization as well as in the configuration of the twisted threads. As mentioned above, the helical structures and the DNA fibrils are localized within the electron-transparent area of the mitochondrial matrix; both are apparently associated. The loss of both structures after DNase digestions, as well as their close association, suggests that they may be similar in nature: possibly circular DNA molecules occurring in either an uncoiled or a supercoiled configuration.

In certain rapidly growing cells the mitochondrial DNA replication is continuous, and gives "polyploid" mitochondria (1) containing a number of DNA molecules. Such a phenomenon presumably occurs in mitochondria of the fir (Pseudotsuga) female gamete, in which Feulgen-positive mitochondria, lacking cristae, have been observed by Chesnoy and Thomas (5). These peculiar mitochondria reach a high cytoplasmic concentration and show large areas of DNA fibrils. The dividing carposporangia of the red alga we have studied grow very rapidly and, in addition, show a number of features which seem rather comparable to those observed by Chesnoy and Thomas (5). These peculiar mitochondria may be interpreted as a stage in the replication of mitochondrial DNA.

The authors express their sincere appreciation to Professor H. Swift for reading and criticizing the manuscript. The technical assistance of Mr. S. Soriente is gratefully acknowledged.