DNA in the Nucleomorph of *Cryptomonas* Demonstrated by DAPI Fluorescence

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DNA has definitely been demonstrated in the nucleomorph of a marine *Cryptomonas* species by combining thin sectioning of Lowicryl K4M-embedded material and DAPI-induced DNA fluorescence.

According to the endosymbiont theory (*cf.* [1]), algal and higher plant plastids as well as eukaryote mitochondria are derived phylogenetically from prokaryotic intracellular symbionts. This hypothesis has recently been tested in several ways (*cf., e.g.,* [2, 3]) and appears now to be well established, especially with respect to plastids.

The plastid envelope normally consists of 2 membranes. The inner one is interpreted, according to the endosymbiont theory, as being derived from the plasma membrane of the prokaryotic cytosymbiont, whereas the outer is assumed to correspond to the membrane of a symbiontophoric vacuole, originally formed by the host cell. Chloroplasts of the Euglenophyta and of chromophytic algae are exceptional in possessing either 3 or even 4 surrounding membranes. To explain this extraordinary situation it has been hypothesized that the plastids of these algae represent remnants of eukaryotic cytosymbionts [4, 5]. It is argued that the cytosymbionts have lost most of their components during further evolution with the exception of the plastids and the plasma membrane. Additionally, in algae where the plastids are surrounded by 4 membranes, the membrane of the symbiontophoric vacuole could have been conserved.

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Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; EM, electron microscope (-scopic); ER, endoplasmic reticulum.

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In this context, the fine structure of unicellular Cryptomonads is of particular interest (*cf.* [6]). Their plastids are surrounded by 4 membranes, the inner ones representing a typical plastid envelope, whereas the 2 outer membranes are referred to as chloroplast ER. Between these two pairs of membranes is a narrow plasmatic compartment containing ribosomes, starch grains, some vesicles, and a tiny "nucleomorph" [4, 5, 7, 8]. There is only one nucleomorph per cell. It contains characteristic inclusions, is covered by a perforated double membrane, and divides just before the onset of nuclear division. As the cryptomonads contain phycobilins, their plastids could be derived from cytosymbiotic red algae, the nucleus of which has been reduced to the nucleomorph during evolution ([4]; for detailed discussions *cf.* [5, 7, 8]). If this assumption is correct, the nucleomorph should contain DNA. In spite of some efforts [5, 7], an unequivocal proof for DNA in the nucleomorph has not yet been published. Using Bernhard's method ([9]; see, however, also [10]) for the EM histochemical demonstration of DNA and RNA, Gillott and Gibbs [5] and Santore [7] came to the conclusion that the nucleomorph "is likely to be a DNA containing organelle" ([8], p. 1058). The very specific and sensitive demonstration of double-stranded DNA by DAPI-induced fluorescence [11] is not applicable to whole-cell preparations as it is impossible to discern the small nucleomorph unequivocally [8]. This difficulty could be circumvented by use of thin sections where identification of organelles, even small ones, is possible since there are no superimpositions. However, DAPI staining of Epon-embedded material in semi-thin sections proved unsuccessful [8].

We therefore tried to apply DAPI to ultrathin sections of Lowicryl-embedded cells of a marine *Cryptomonas* species [12] the nucleomorph of which is tightly surrounded by the chloroplast pyrenoid (Fig. 1). Lowicryl K4M [13] is a hydrophilic resin which can be photopolymerized by UV at low temperature and provides improved preservation of antigenicity, as the samples need not be fully dehydrated. Ultrathin sections of Lowicryl-embedded *Cryptomonas* cells showed bright DAPI fluorescence of the nuclear chromatin (but not of the nucleolus), the several chloroplast nucleoids, and of distinct areas of the nucleomorph (Fig. 2a). The nucleomorph can easily be identified by subsequent observation of the same section in the EM (Figs. 2b–2d).

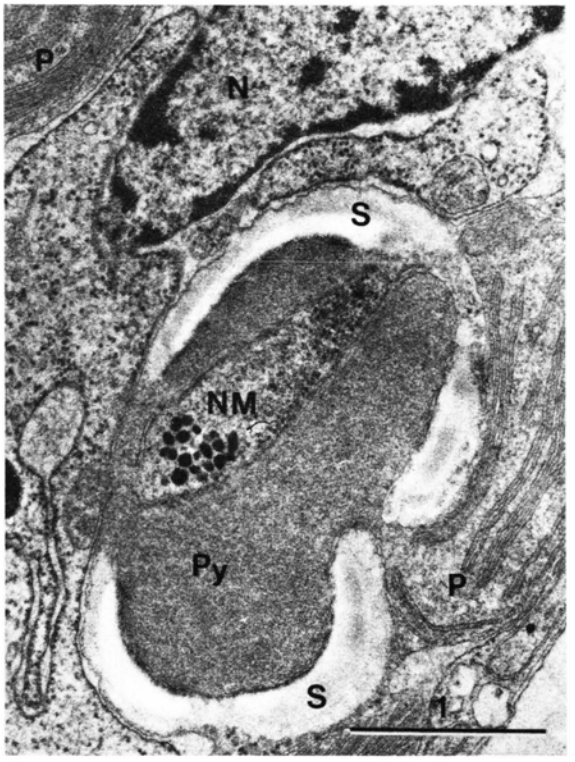
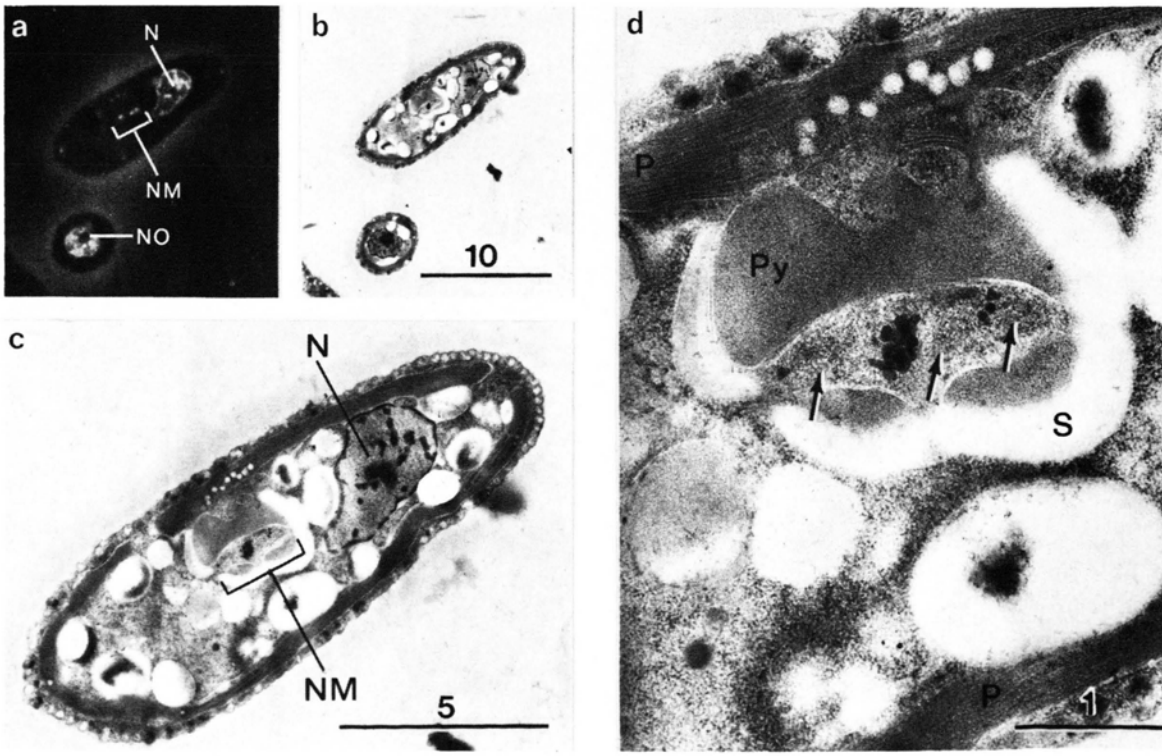


Fig. 1. Nucleomorph surrounded by pyrenoid in *Cryptomonas* spec. Cells were fixed in cacodylate-buffered (pH 7.4) glutaraldehyde followed by osmium tetroxide (same buffer) and *en bloc* stained by uranyl acetate. Dehydration, embedding in Spurr's low viscosity resin, double-staining of ultrathin sections was done according to conventional methods. Py, pyrenoid; NM, nucleomorph; N, nucleus; P, plastid; S, starch; Bar: 1 μm.

Fig. 2. Localization of DNA in the nucleomorph of *Cryptomonas* by combination of DAPI-fluorescence and electron microscopy. For this purpose, the cells were fixed in 4% formaldehyde (in order to stabilize DNA; *cf.* [14]), 0.5% glutaraldehyde, 0.1 M phosphate buffer (pH 7.4), and 0.5 M sucrose for 3 h at room temperature. After washing in buffer the cells were dehydrated and embedded in Lowicryl K4M at −30 °C [13]. Ultrathin sections (120–150 nm) were incubated in an aqueous DAPI solution (0.1 μg/ml) for 8 min on Formvar-coated nickel EM-grids. After a brief wash the grids were mounted in 50% glycerol on glass slides for light-microscopical observation using a Zeiss IM 35 epifluorescence microscope equipped with Neofluar optics and a HBO 50 lamp. Photographs were taken on Kodak Tri-X pan (400 ASA) film using the filter combination BP 365/FT 395/LP 397. The grids were then washed, stained with 2% aqueous uranyl acetate for 5 min, poststained with Pb citrate, and dried. Observation in a Zeiss EM 10. (a) Ultrathin section of cells showing DAPI fluorescence in the nucleus (N) and nucleomorph (NM). Note absence of fluorescence in the nucleolus (NO). – (b), (c), (d) Electron micrographs of the same section at equal (b) and higher magnifications. Bars: (b) 10 μm, (c) 5 μm, (d) 1 μm. Letters as in Fig. 1. Areas corresponding to fluorescent spots in the nucleomorph are marked by arrows (d).



DNase I, when applied to proteinase K treated sections, diminished considerably the fluorescence intensity of all DNA containing structures.

In conclusion, using this new combination of DAPI fluorescence histochemistry on Lowicryl-embedded material with EM identification of the fluorescing cellular structures it was possible to show that DNA is in fact contained in the nucleomorph of cryptomonads. This result supports the hypothesis that the plastids of these flagellates have evolved from a eukaryotic cytosymbiont [4, 5].

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Note added in proof: After submission of the present paper, a publication by M. Ludwig and S. P. Gibbs on the same subject appeared in Protoplasma **127,** 9–20 (1985). These authors, by use of DAPI fluorescence, arrived at the same conclusions concerning the presence od DNA in the nucleomorph as presented here.

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