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Ultrastructural localization of DNA in two Cryptomonas species by use of a monoclonal DNA antibody

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The distribution and subcellular localization of DNA in the unicellular alga Cryptomonas has been investigated electron-microscopically by indirect immunocytochemistry, using a monoclonal DNA antibody and a gold-labeled secondary antibody. This technique proved to be very sensitive and entirely specific. DNA could be demonstrated in four different compartments (nucleus, nucleomorph, plastid, and mitochondrion).

Within the plastid, DNA is concentrated in stroma regions that are localized preferentially around the center of the organelle. The mitochondrion contains several isolated DNA-containing regions (nucleoids). Within the nucleus, most of the DNA is localized in the 'condensed' chromatin. DNA was also detectable in small areas of the nucleolus, whereas the interchromatin space of the nucleus appeared almost devoid of DNA. Within the nucleomorph, DNA is distributed inhomogeneously in the matrix. DNA could furthermore be detected in restricted areas of the 'fibrillogranular body' of the nucleomorph, resembling the situation encountered in the nucleolus.

The presence of DNA and its characteristic distribution in the nucleomorph provide additional, strong evidence in favour of the interpretation of that organelle as the residual nucleus of a eukaryotic endosymbiont in Cryptomonas.

Introduction

Presently, there are two alternative hypotheses on the phyletic origin of plastids and mitochondria in eukaryotic cells (eucytes). Whereas an endosymbiotic origin of DNA-containing organelles has been postulated by several authors ('endosymbiont hypothesis', cf. [31]), others favour a non-symbiotic, autogenous origin (cf. [6]). In recent years, a growing body of evidence, mainly from biochemical and molecular biological studies, has accumulated in support of the endosymbiont hypothesis. In the case of plastids especially, this hypothesis now seems well established [18].

The endosymbiont hypothesis maintains, in its original form, that plastids are derived from photosynthetic prokaryotes that became incorporated as cytosymbionts into eucytes already containing mitochondria. Such an interpretation may well apply to the plastids of red algae as well as chlorophytes and all the higher plants, but not to other plants (algae), the plastids of which are surrounded by more than two envelope membranes. In these cases, it has been suggested that photosynthetic, unicellular eukaryotes were incorporated into zooflagellates as cytosymbionts and, during further evolution, became gradually reduced to 'plastids' (cf. [16, 46]). An intermediary state in the phyletic reduction of cytosymbiotic algal cells to 'plastids' in zooflagellates is possibly presented by the unicellular cryptomonads. The plastids of cryptomonads are surrounded by four membranes, the inner two corresponding to the ordinary plastid envelope, the outer two being referred to as 'periplastidal ER'. Between the two pairs of membranes is a narrow plasmatic compartment, supposedly the remnant of the cytoplasm of a former endosymbiont. This compartment contains, amongst ribosomes and starch deposits, an organelle surrounded by two membranes with pores that presumably represents a residual nucleus. Consequently, it has been termed 'nucleomorph' [20]. It contains a few 'dense particles' and a 'fibrillogranular body', the latter possibly being the remains of a nucleolus [17]. In the cryptomonad cell, the number of nucleomorphs is always the same as the number of plastids. Depending on the particu-

Abbreviations. DP Electron-dense particles. — E Ejectisome. — FG Fibrillogranular body. — G Gullet. — M Mitochondrion. — N Nucleus. — Nm Nucleomorph. — No Nucleolus. — P Plastid. — Py Pyrenoid. — S Starch.

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lar species, this number is either one or two [40]. The nucleomorph is located either within an invagination of the plastid's pyrenoid, or free in the space between the plastid envelope and the periplastidal ER [17]. The nucleomorph divides immediately before the onset of nuclear and cell division [32, 34].

If the interpretation of the nucleomorph as the residual nucleus of a former eukaryotic endosymbiont is correct, the nucleomorph might be expected to contain DNA. In fact, DNA in the nucleomorph could recently be demonstrated unequivocally by DAPI fluorescence [29, 23]. Earlier attempts to detect DNA within the nucleomorph and to trace its finer distribution by electron microscopic methods proved either unsuccessful or led to ambiguous results [17, 39].

There are several EM cytochemical methods for studying DNA distribution at a subcellular and even suborganelle level (cf. [35]). Recently, a DNase gold method has been developed by Bendayan [2] for the fine structural localization of DNA. However, this method could not be successfully applied to Cryptomonas (unpublished own results). By use of a monospecific, monoclonal DNA antibody we could now precisely localize the DNA in the four DNA-containing organelles of two different Cryptomonas species. This enabled us to compare DNA distribution in the nucleomorph with that of the cell nucleus. Furthermore, new information could be obtained regarding DNA topology of plastids and mitochondria.

Materials and methods

Cell cultures

Cryptomonas ovata was obtained from the Sammlung von Algenkulturen, Göttingen/FRG, and was cultured on desmidiacean medium [44]. Cryptomonas sp. was a gift of Dr. P. Emschermann, University of Freiburg/FRG, and was grown on f/2 medium [21]. Both cultures were maintained at 21 °C with a 12 h light/12 h dark cycle. Cells were harvested for fixation 20 days after inoculation.

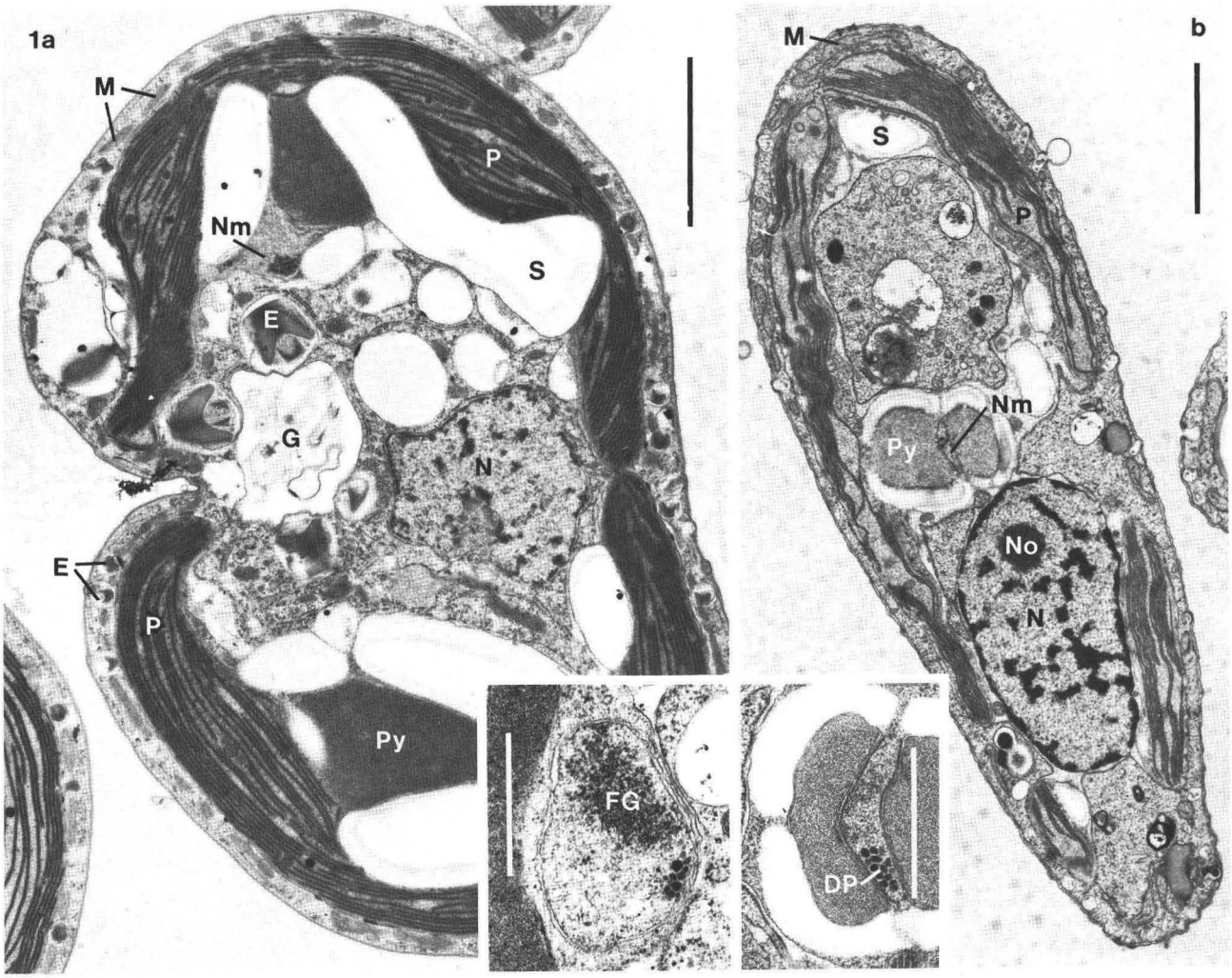


Fig. 1. Overview of Cryptomonas ovata (**a**) and Cryptomonas sp. (**b**) after glutaraldehyde/OsO_4 fixation and Epon-embedding. — *Insets* show the fine structure of nucleomorphs at higher magnification. — Bars 2 μm (**a, b**), 1 μm (*insets*).

Conventional electron microscopy

For fixation and washing the cells were collected by centrifugation at 500*g* for 5 min. All fixation and washing steps were performed at room temperature.

C. ovata was prefixed in the culture medium by adding glutaraldehyde and cacodylate buffer, *p*H 7.4, to final concentrations of 0.5% and 10 mM. After 30 min the cells were collected, fixed in 3% glutaraldehyde + 50 mM cacodylate buffer, *p*H 7.4, for 3 h and washed five times in buffer. C. sp. was fixed in 3% glutaraldehyde + 50 mM cacodylate buffer, *p*H 7.4, osmotically balanced with 0.5 M sucrose for 3 h and washed five times in buffer containing decreasing amounts of sucrose. Cells of both species were postfixed in 2% OsO_4 in buffer for 1 h. After washing in buffer, embedding in low-gelling-temperature agarose and staining en bloc in 2% aqueous uranyl acetate for 1 h, the cells were dehydrated in a graded ethanol series and embedded in Epon 812. Sections (Ultracut microtome, Reichert-Jung/FRG) were poststained with uranyl acetate and lead citrate. Micrographs were taken with a ZEISS EM 10 CR electron microscope.

EM immunocytochemistry

C. ovata was prefixed as described above. Thereafter the cells were further fixed in 4% formaldehyde (freshly prepared from paraformaldehyde), 0.5% glutaraldehyde in 50 mM phosphate buffer, *p*H 7.4, for 3 h and washed in buffer. C. sp. was fixed in the same medium as C. ovata but containing 0.5 M sucrose and washed in a decreasing sucrose series in buffer. Cells of both species were embedded in low-gelling-temperature agarose at 35 °C. Dehydration and embedding in Lowicryl K4M followed the standard procedure of Carlemalm et al. [5]. The resin was UV-polymerized at −30 °C for 24 h and at room temperature for a further 5 days. Pale gold sections were cut on the ultramicrotome and mounted on carbon-reinforced Formvar-coated 200 mesh hexagonal nickel grids.

The hybridoma clone AK 30-10 producing monoclonal antibodies (IgM) to DNA was the result of a fusion of cells of the mouse myeloma line Ag 8.653 with the spleen cells of a BALB/c mouse that was previously immunized with the high salt and detergent resistant material of a rat hepatoma. IgM immunoglobulins were purified from ascites fluid by gel filtration on Sephacryl S 300 columns (Pharmacia, Uppsala/Sweden) with 0.5 M NaCl, 50 mM Tris-HCl, *p*H 7.5, as elution buffer at 4 °C. The early eluting IgM fractions were concentrated by vacuum dialysis (Sartorius, Göttingen/FRG) against phosphate-buffered saline (PBS) to a final concentration of 1 mg/ml. The antibody binds to double and single-stranded DNA but not to RNA. A detailed account on this antibody, including binding studies by competitive ELISA-tests with a variety of synthetic oligonucleotides and natural nucleic acids, will be presented elsewhere [42].

For labeling of the sections the following protocol was applied (all steps were performed at room temperature): 5 min bidistilled water (b.w.); 10 min PBSFT (PBS plus 5% *f*etal calf serum, 0.1% *T*ween 20); 1 h monoclonal DNA antibody in PBSFT (10 µg/ml); twice 10 min PBSFT; twice 10 min PBSBT (PBS plus 1% *B*SA, 0.1% *T*ween 20); 1 h GAMIgM (diluted 75-fold with PBSBT); four times 10 min PBSBT; three times 5 min b.w.; air drying; 5 min b.w.; 8 min 2% aqueous uranyl acetate; three times 1 min b.w.; 1 min lead citrate; three times 1 min b.w.; jet wash with b.w.; finally the sections were air dried.

Three controls for labeling were carried out. First, the monoclonal DNA antibody was omitted. Second, the monoclonal DNA antibody was preincubated with plasmid DNA (pBR 328) in PBSFT for 1 h. Third, the grids were preincubated at 30 °C for different lengths of time (see Results) with 1 mg/ml DNAse I (Serva, Heidelberg/FRG) in PBS plus 7 mM $MgCl_2$. After every 2 h the reaction mixture was renewed.

Results

Cryptomonas cell fine structure

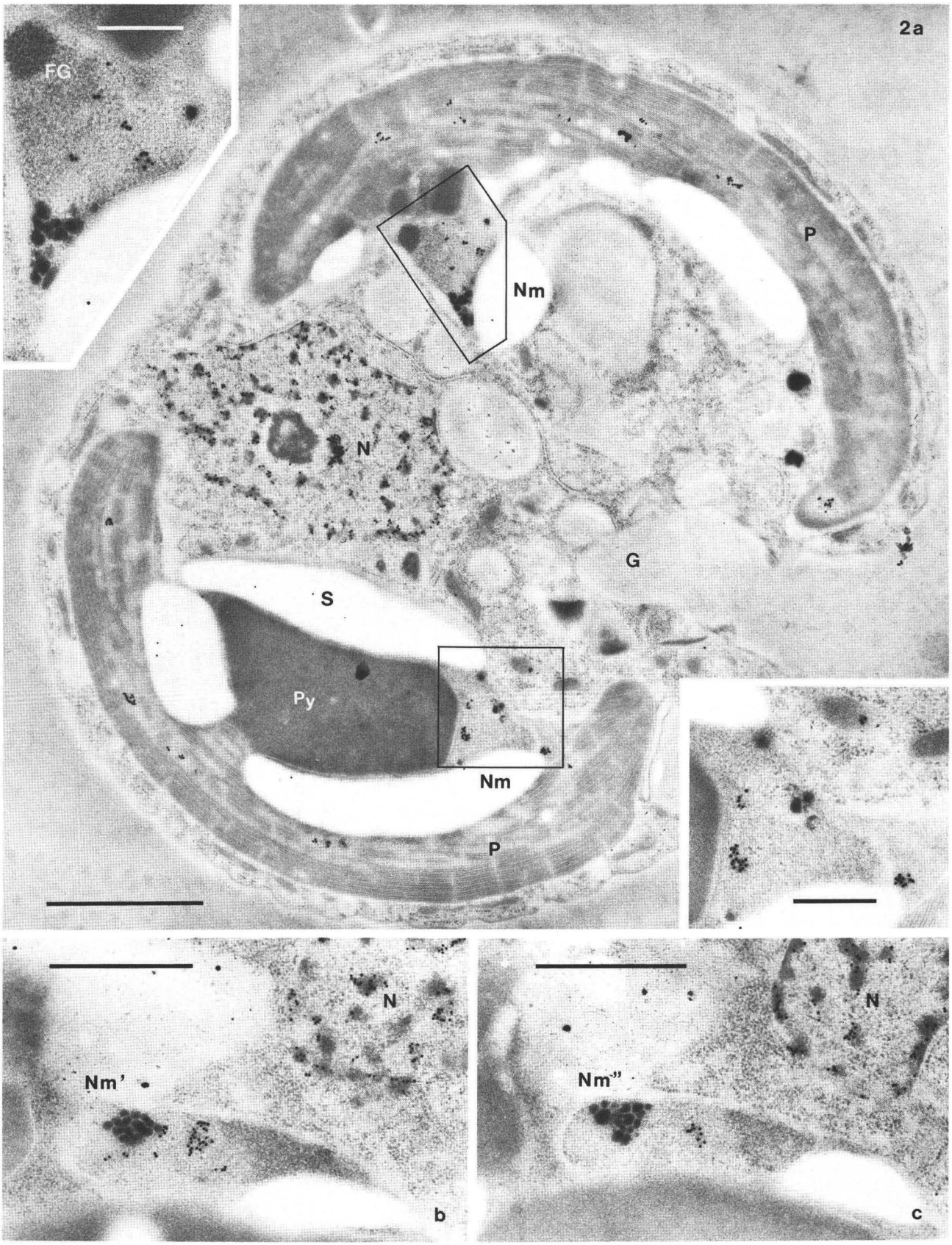
Two Cryptomonas species, distinctively different in fine structure and physiology, were chosen for our study. C. ovata is a unicellular freshwater alga with two plastids and two corresponding nucleomorphs per cell. The nucleomorphs are located beneath the inner, concave side of the plastids (Fig. 1a). The second Cryptomonas species (C. sp.) has not been identified. Its natural habitat is seawater, and there is only one nucleomorph per cell, located in an invagination of the pyrenoid (Fig. 1b). The nucleomorphs of both species are composed of an envelope of two membranes with pores, an amorphous ground matrix (GM) with a 'fibrillogranular' region (FG) and a few ± spherical 'dense particles' (DP). Both the FG and the DP are heavily contrasted by uranyl acetate (*insets*, Fig. 1) which indicates that both these components contain nucleic acids. In both species, the nucleus contains one nucleolus. There is no nucleolus-associated heterochromatin. Condensed chromatin can be observed (particularly well in C. sp.) as electron-dense domains scattered throughout the nucleus and accumulated at the inner side of the nuclear envelope. The nonplasmatic perinuclear space is continuous with the periplastidal ER that, in turn, surrounds the whole plastid. The plastids contain, in their central part, some matrix domains free of thylakoids. Many extrusive organelles ('ejectisomes') are situated in the peripheral plasma of the cells, and there is also one large mitochondrion (Fig. 1).

DNA localization

In Figure 2, the results of DNA immunolabeling in C. ovata are shown. The survey (Fig. 2a) demonstrates the typical clustering of gold particles at places of high local DNA concentration. There is only faint background labeling, confined primarily to starch deposits. Both nucleomorphs are, however, strongly labeled (*insets* Fig. 2a). The distribution of the label is inhomogeneous, the fibrillogranular regions being only seldom labeled, and the DP never. DNA-positive areas of the GM could invariably be followed through successive serial sections (Figs. 2b, c). This points to an inhomogeneous distribution of DNA in the ground matrix of the nucleomorph.

Similar results were obtained with C. sp. (Fig. 3). In the nucleus, regions of condensed chromatin are heavily labeled (Fig. 3a), whereas only a few gold particles are found associated with interchromatin areas (some of which could, moreover, belong to tangentially cut, and hence not discernible, condensed chromatin). Nucleomorphs are in most cases strongly labeled (Fig. 3a), the gold particles be-

Fig. 2. Immunocytochemical localization of DNA on Lowicryl K4M sections of C. ovata. — **a.** Overview of a whole cell with the two nucleomorphs (see also *insets*). — Note the nonrandom distribution of gold particles in the nucleus, nucleomorphs and plastids. — **b, c.** Serial sections of a nucleomorph with corresponding regions immunolabeled. — Bars 2 µm (**a**), 1 µm (**b, c**), *insets* (**a**) 0.5 µm.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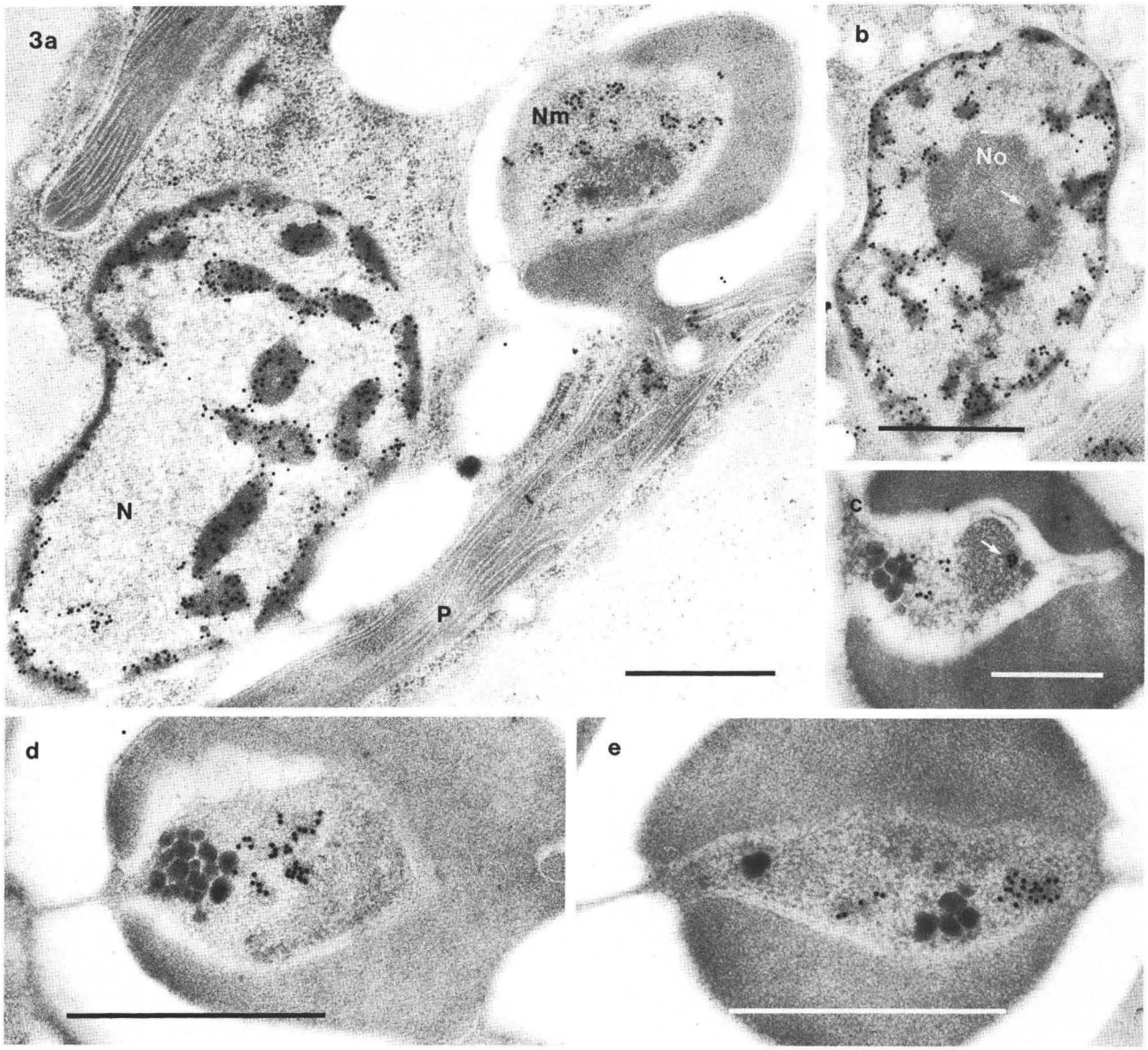

Fig. 3. Immunocytochemical labeling of DNA on Lowicryl K4M sections of C. sp. — **a.** Overview of the nucleus and nucleomorph. Only a small region of the nucleolus (**b**) and of the fibrillogranular body (**c**) are labeled (*arrows*). The distribution of the gold particles in the ground matrix of the nucleomorph is not homogeneous (**d, e**). — Bars 1 μm (**a, b, d, e**), 0.5 μm (**c**).

ing distributed inhomogeneously mainly throughout the GM (Figs. 3a, d, e). As in C. ovata, DP are never labeled. Sometimes, small areas within the FG of the nucleomorph are also heavily labeled (Fig. 3c). Corresponding observations could again be made for the nucleolus of the cell nucleus (Fig. 3b).

Besides the labeling of nuclei and nucleomorphs, gold particles could also be found over limited regions of the plastids and mitochondria. In plastids, regions of stroma matrix in the inner part of the organelle are densely labeled (Figs. 2, 3, 4b). Not all of the mitochondrial profiles are labeled in sections (cf. Figs. 2 and 3 with Fig. 4a), suggesting that the mitochondrion is comprised of several 'nucleoids'.

Specificity of DNA labeling

The specificity of DNA labeling on sections by the immunogold technique applied in this study was tested in several ways. There was virtually no labeling if the primary antibody was either omitted or saturated by incubation with protein-free DNA prior to its application to sections (Figs. 5a, c). If the antigen was specifically removed from the surface of the sections by rinsing them in a solution of DNase I, the labeling was gradually diminished and eventually abolished. After a treatment with DNase I for 2 to 4 h, nucleomorphs (Fig. 5d), mitochondria, and plastids could no longer be labeled. Chromatin DNA of the nucleus proved to be more resistant, particularly in the case

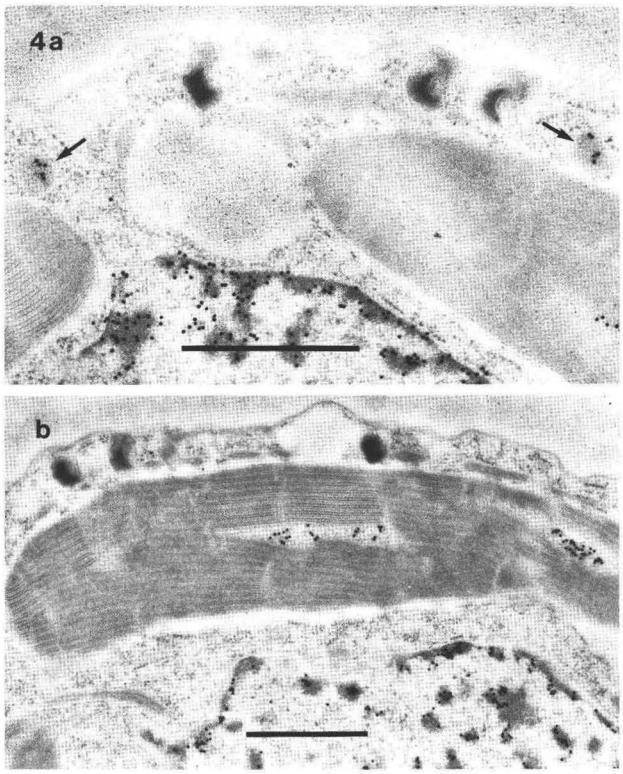

Fig. 4. Localization of DNA in profiles of the mitochondrion (**a**) and plastid (**b**) of C. ovata. Most of the stromal regions of the plastid are labeled whereas only a few cross sections of the mitochondrion show immunostaining (*arrows*). — Bars 1 μm.

of the denser chromatin of C. sp. Even so, after 4 h treatment with DNase I only very few gold particles appeared to be associated with the areas of condensed chromatin (Fig. 5b).

Discussion

EM cytochemistry of DNA

At the beginning of our investigation of DNA topology in Cryptomonas cells, and DNA distribution in nuclei as compared to that in nucleomorph, the DNase I-gold technique of Bendayan [2] appeared to be the most appropriate procedure. This method has been claimed to be specific for DNA [3], and it had already been employed successfully by several groups [7]. However, in Cryptomonas not only the nucleus was heavily labeled by this method but also ejectisomes (results not shown here). Ejectisomes are, however, known to consist of proteins [33] and presumably they do not contain DNA. On the other hand, neither mitochondria nor plastids were labeled in Cryptomonas, although these organelles definitely do contain DNA. Another point speaking against the DNase I-gold technique as a specific cytochemical means for localizing DNA is the following. Actin is known to be a strong inhibitor of DNase I [27]. In fact, DNase I exhibits an extreme affinity for G-actin (binding constant $5 \times 10^8\ \text{M}^{-1}$; for F-actin: $1.2 \times 10^4\ \text{M}^{-1}$; [30]). As Wang and Goldberg [45] have shown, it is possible to visualize actin microfilaments in nonmuscle cells, either directly by rhodamine-DNase I conjugates, or by indirect immunofluorescence (DNase I + fluorescent DNase I antibody). Moreover, actin is purportedly present in many (all?) nuclei [28]. The nuclear actin occurs mainly in the G form showing high affinity for DNase I [9, 43]. The concentration of actin in nuclei is, in many cases, considerable. For example, in amphibian oocyte nuclei it reaches 3 to 4 $\text{mg} \cdot \text{ml}^{-1}$ [10, 26]. In Physarum, the local actin concentration in the nuclei may exceed that of the cytoplasm by one order of magnitude [25]. It therefore cannot be excluded that with the DNase I-gold technique not only DNA but also actin is being localized [38].

Another method for detecting DNA at the EM level is radioautography. However, only actively replicating DNA can be labeled with radioactive precursors, and due to the long exposure times, a redistribution of labeled products cannot be fully excluded [1]. Furthermore, the radioautographic resolution is below that of the EM. We therefore decided to make use of a newly developed monoclonal DNA antibody [42] as the primary antibody in indirect immunocytochemistry of DNA. The high specificity of the monoclonal antibody has been established by several control experiments, described in Materials and methods.

DNA topology of nuclei

In the present study, it was shown that most of the nuclear DNA is located in the regions of condensed chromatin and in the nucleolus organizer region, whereas the 'dispersed' chromatin appeared to be almost free of DNA. Corresponding results have been obtained, by use of a Feulgen-type reaction (osmium-ammine-SO_2 [11], cf. [15]), with interphase nuclei of Euglena [36] and, amongst many others, including rat hepatocyte nuclei [13] (cf. also [35]). There is growing evidence for a distinct functional organization of interphase nuclei [22]. DNA replication, as well as transcription, seem to take place preferentially at the irregular boundaries between condensed and dispersed chromatin [37, 24]. Conceivably, dispersed chromatin comprises only the relatively small amount of activated DNA sequences. If so, nuclear DNA topology should strongly depend on the physiological state of the respective cell. In our case, the algae were just entering the stationary phase when they were fixed. In this state, both replication and transcription are at a very low level. This would mean that nearly all chromatin was in a condensed condition, leaving the rest of the nuclear compartment ('dispersed chromatin') almost devoid of DNA.

DNA topology of the nucleomorph

The occurrence of DNA in the nucleomorph of cryptomonads, demonstrated quite recently by DAPI fluorescence [29, 23], has been corroborated in the present study by an indirect method. Furthermore, the fine structural localization of DNA in the nucleomorph shows striking similarities of DNA distribution in the nucleus. Most of the nucleomorph DNA is distributed inhomogeneously in the ground matrix of the organelle. The fibrillogranular region

of the nucleomorph has been proposed by Gillott and Gibbs [17] to correspond to a nucleolus. Santore [39], after employing Bernhard's uranyl acetate/EDTA technique [4], reported DNA to be concentrated particularly in this region of the nucleomorph. On the other hand, using the same technique, Gillott and Gibbs [17] were not able to find DNA in the fibrillogranular body. The evidence obtained by these authors implied rather that the fibrillogranular region contained RNA. We found DNA in small areas of the fibrillogranular region. This corresponds exactly to the situation in the nucleolus, where the DNA of the nucleolus organizer region is confined to meandering, narrow 'lacunae' within a structure otherwise dominated by ribonucleoprotein. It is possible that, within the fibrillogranular region, the synthesis of rRNA takes place as a prerequisite for the formation of the ribosomes of the periplastidal compartment which, as judged from their size, are of the eukaryotic type [17].

The composition and function of the 'dense particles' in the nucleomorph remain unclear. According to Gillott and Gibbs [17] these particles might be RNA virions. Morrall and Greenwood [34] considered the possibility that the dense particles might be condensed chromatin. We were, however, not able to detect DNA in these particles. Accordingly, they seem unlikely to be chromatin of any kind.

The nucleoids of plastids and mitochondria

On the basis of DAPI fluorescence data, Coleman [12] reported on the distribution of DNA in the plastids of about one hundred algal species. The identification of plastid nucleoids proved difficult, however, in the case of tiny cryptomonads with their additional, DNA-containing nucleomorph. Nevertheless, Coleman's results are entirely consistent with ours. DNA is contained in almost every region of the plastids from which thylakoids are excluded, and in which stroma material is accumulated. Areas of this kind ('nucleoids') are scattered throughout the whole plastid, although most of them are located more or less centrally.

Every Cryptomonas cell contains one large mitochondrion which, according to Santore and Greenwood [41], forms an extended network. Since DNA could be detected, in our study, in only some of the mitochondrial profiles in sections, it can be concluded that there are several nucleoids per mitochondrion.

Nucleomorphs and the endosymbiont hypothesis

The results presented here supply further support for the opinion, first expressed by Greenwood [19], that the nucleomorph of cryptomonads might represent a residual nucleus of a eukaryotic endosymbiont that, during long-lasting phyletic coevolution, became reduced almost to its

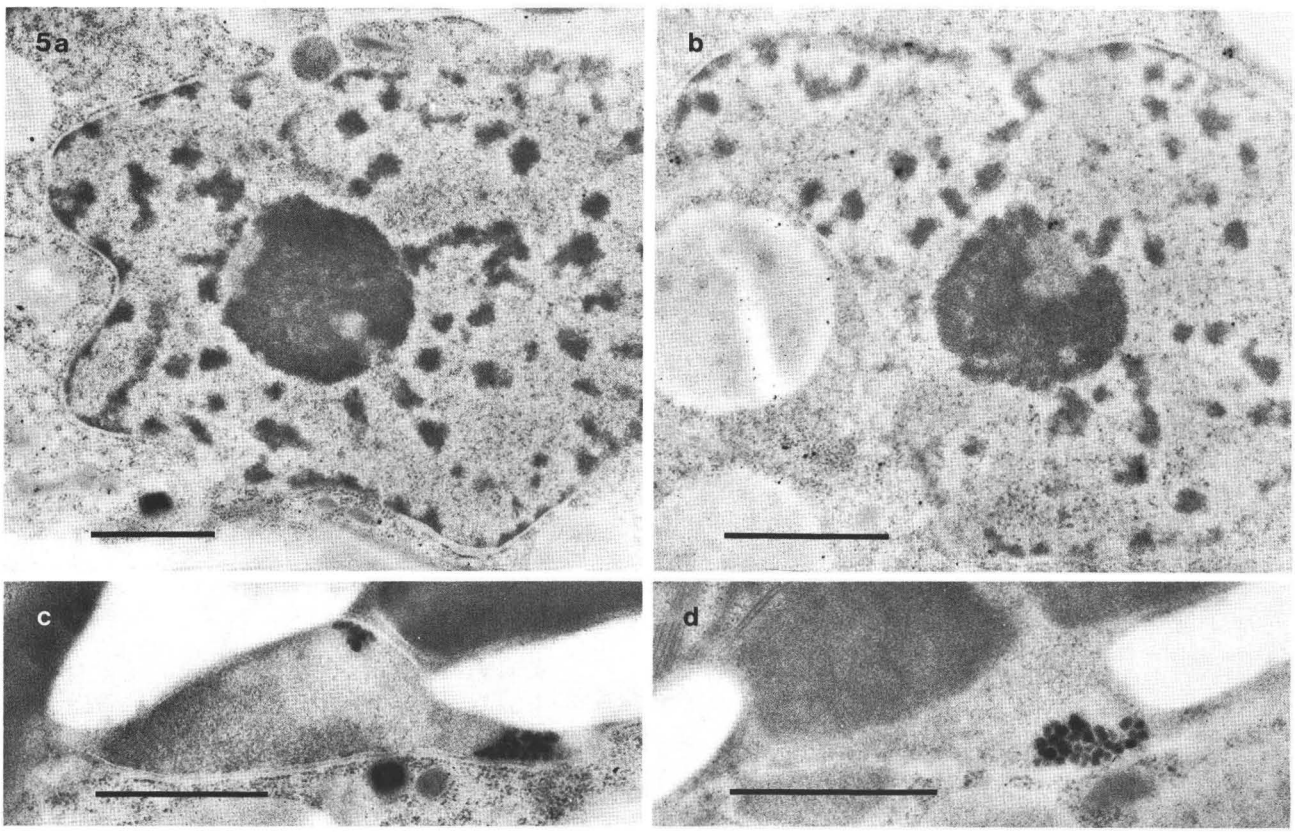

Fig. 5. Labeling controls on Lowicryl K4M sections of C. ovata showing nuclei (**a, b**) and nucleomorphs (**c, d**). Preincubation of the DNA antibody with plasmid DNA (pBR 328; **a, c**). DNase I treatment of the sections for 4 h prior to labeling (**b, d**); only a few gold particles are visible over the condensed chromatin of the nucleus. — Bars 1 μm.

plastid(s) in the cells of zooflagellates. Whereas in other Chromophyta (sensu Christensen [8]) only plastids and a single surrounding membrane (presumably the plasma membrane of the cytosymbiont) have been left over by this gradual adaptive reduction, some members of the Chromophyta still possess a few additional structures contained in a narrow periplastidal compartment (e.g. 'periplastidal reticulum' [14]). In cryptomonads, however, some remains of the symbiont's cytoplasm *and nucleus* apparently have been retained. This would mean that intermediary states in intracellular adaptation of photosynthetic cytosymbionts have been preserved in some 'algae', so that transitional stages of evolution could be investigated in contemporary organisms which provide us with 'living fossils' at the cellular level.

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References

[1] Angelier, N., M. Bouteille, R. Charret, J. J. Curgy, E. Delain, S. Fakan, M. Geuskens, M. Guelin, J. C. Lacroix, M. Laval, G. Steinert, S. Van Assel: Detection of nucleic acids by means of ultrastructural autoradiography. J. Microsc. Biol. Cell. **27,** 215–230 (1976).

[2] Bendayan, M.: Ultrastructural localization of nucleic acids by use of enzyme-gold complexes. J. Histochem. Cytochem. **29,** 531–541 (1981).

[3] Bendayan, M., E. Puvion: Ultrastructural localization of nucleic acids through several cytochemical techniques on osmium-fixed tissues. J. Histochem. Cytochem. **32,** 1185–1191 (1984).

[4] Bernhard, W.: A new staining procedure for electron microscopical cytology. J. Ultrastruct. Res. **27,** 250–265 (1969).

[5] Carlemalm, E., R. M. Garavito, W. Villiger: Resin development for electron microscopy and an analysis of embedding at low temperature. J. Microscopy **126,** 123–143 (1982).

[6] Cavalier-Smith, T.: The origin of nuclei and of eukaryotic cells. Nature **256,** 463–467 (1975).

[7] Charest, P. M., F. Bergeron, J. G. Lafontaine: Ultrastructural localization of DNA and RNA in Allium porrum interphase cells by means of nuclease-gold complexes. Histochem. J. **17,** 957–966 (1985).

[8] Christensen, T.: The gross classification of algae. In: D. F. Jackson (ed.): Algae and Man. pp. 59–64. Plenum Press. New York, London 1964.

[9] Clark, T. G., R. W. Merriam: Diffusible and bound actin in nuclei of Xenopus laevis oocytes. Cell **12,** 883–981 (1977).

[10] Clark, T. G., J. L. Rosenbaum: An actin filament matrix in hand isolated nuclei of X. laevis oocytes. Cell **18,** 1101–1108 (1979).

[11] Cogliati, R., A. Gautier: Mise an évidence de l'ADN et des polysaccharides à l'aide d'un nouveau réactif "de type Schiff". C. R. Acad. Sci. (Paris) Sér. D **276,** 3041–3044 (1973).

[12] Coleman, A. W.: Diversity of plastid DNA configuration among classes of eukaryote algae. J. Phycol. **21,** 1–16 (1985).

[13] Derenzini, M., A. Viron, F. Puvion-Dutilleul: The Feulgen-like osmium-ammine reaction as a tool to investigate chromatin structure in thin sections. J. Ultrastruct. Res. **80,** 133–147 (1982).

[14] Falk, H., H. Kleinig: Feinbau und Carotinoide von Tribonema (Xanthophyceae). Arch. Mikrobiol. **61,** 347–362 (1968).

[15] Gautier, A.: Ultrastructural localization of DNA in ultrathin tissue sections. Int. Rev. Cytol. **44,** 113–191 (1976).

[16] Gibbs, S. P.: The chloroplasts of some algal groups may have evolved from endosymbiotic eukaryotic algae. In: J. F. Fredrick (ed.): Origins and Evolution of Eukaryotic Intracellular Organelles. Vol. 361. pp. 193–208. Ann. New York Acad. Sci. 1981.

[17] Gillott, M. A., S. P. Gibbs: The cryptomonad nucleomorph: its ultrastructure and evolutionary significance. J. Phycol. **16,** 558–568 (1980).

[18] Gray, M. W., W. F. Doolittle: Has the endosymbiont hypothesis been proven? Microbiol. Rev. **46,** 1–42 (1982).

[19] Greenwood, A. D.: The Cryptophyta in relation to phylogeny and photosynthesis. In: J. V. Sanders, D. J. Goodchild (eds.): 8th Int. Congr. Electron Microsc. Vol. 2. pp. 566–567. Canberra 1974.

[20] Greenwood, A. D., H. B. Griffiths, U. J. Santore: Chloroplasts and cell compartments in Cryptophyceae. Br. Phycol. J. **12,** 119 (1977).

[21] Guillard, R. R. L.: Culture of phytoplankton for feeding marine invertebrates. In: W. L. Smith, M. H. Chanley (eds.): Culture of Marine Invertebrate Animals. pp. 29–60. Plenum Press. New York 1975.

[22] Hancock, R., T. Boulikas: Functional organization in the nucleus. Int. Rev. Cytol. **79,** 165–214 (1982).

[23] Hansmann, P., H. Falk, P. Sitte: DNA in the nucleomorph of Cryptomonas demonstrated by DAPI fluorescence. Z. Naturforsch. **40c,** 933–935 (1985).

[24] Hutchison, N., H. Weintraub: Localization of DNase I-sensitive sequences to specific regions of interphase nuclei. Cell **43,** 471–482 (1985).

[25] Jockusch, B. M., M. Becker, I. Hindennach, H. Jockusch: Slime mold actin: homology to vertebrate actin and presence in the nucleus. Exp. Cell Res. **89,** 241–246 (1974).

[26] Krohne, G., W. W. Franke: A major soluble acidic protein located in nuclei of diverse vertebrate species. Exp. Cell Res. **129,** 167–189 (1980).

[27] Lazarides, E., U. Lindberg: Actin is the naturally occurring inhibitor of deoxyribonuclease I. Proc. Natl. Acad. Sci. USA **71,** 4742–4746 (1974).

[28] LeStourgeon, W. M.: The occurrence of contractile proteins in nuclei and their possible functions. In: H. Busch (ed.): The Cell Nucleus. Vol. 6. pp. 305–326. Academic Press. New York 1978.

[29] Ludwig, M., S. P. Gibbs: DNA is present in the nucleomorph of cryptomonads: further evidence that the chloroplast evolved from a eukaryotic endosymbiont. Protoplasma **127,** 9–20 (1985).

[30] Mannherz, H. G., R. S. Goody, M. Konrad, E. Nowak: The interaction of bovine pancreatic deoxyribonuclease I and skeletal muscle actin. Eur. J. Biochem. **104,** 367–379 (1980).

[31] Margulis, L.: Origin of Eukaryotic Cells. Yale University Press. New Haven 1970.

[32] McKerracher, L., S. P. Gibbs: Cell and nucleomorph division in the alga Cryptomonas. Can. J. Bot. **60,** 2440–2452 (1982).

[33] Morrall, S., A. D. Greenwood: A comparison of the periodic substructure of the trichocysts of the Cryptophyceae and Prasinophyceae. BioSystems **12,** 71–83 (1980).

[34] Morall, S., A. D. Greenwood: Ultrastructure of nucleomorph division in species of Cryptophyceae and its evolutionary implications. J. Cell Sci. **54,** 311–328 (1982).

[35] Moyne, G.: Methods in ultrastructural cytochemistry of the cell nucleus. Prog. Histochem. Cytochem. **13,** 1–72 (1980).

[36] Moyne, G., O. Bertaux, E. Puvion: The nucleus of Euglena. I. An ultracytochemical study of the nucleic acids and nucleoproteins of synchronized Euglena gracilis Z. J. Ultrastruct. Res. **52,** 362–376 (1975).

[37] Puvion, E., G. Moyne: In situ localization of RNA structures. In: H. Busch (ed.): The Cell Nucleus. Vol. 8. Part A. pp. 59–115.

Academic Press. New York, London, Toronto, Sydney, San Francisco 1981.
[38] Raska, I., B. L. Armbruster, M. Jira, I. Hana, J. Kaslik, J. Rovensky, K. Smetana: Ultrastructural localization of DNA in tissue culture cell nuclei by means of enzyme-gold and autoimmune sera-protein-A-gold techniques. Biol. Cell **48,** 211–214 (1983).
[39] Santore, U. J.: The distribution of the nucleomorph in the Cryptophyceae. Cell Biol. Int. Rep. **6,** 1055–1063 (1982).
[40] Santore, U. J.: Some aspects of taxonomy in the Cryptophyceae. New Phytol. **98,** 627–646 (1984).
[41] Santore, U. J., A. D. Greenwood: The mitochondrial complex in Cryptophyceae. Arch. Microbiol. **112,** 207–218 (1977).
[42] Scheer, U., K. Messner, I. Raska, P. Hansmann, H. Falk, R. Thomae, W. W. Franke: Manuscript in preparation (1986).
[43] Scheer, U., H. Hinssen, W. W. Franke, B. M. Jockusch: Microinjection of actin-binding proteins and actin antibodies demonstrates involvement of nuclear actin in transcription of lampbrush chromosomes. Cell **39,** 111–122 (1984).
[44] Schlösser, U. G.: Sammlung von Algenkulturen: List of strains. Ber. Dtsch. Bot. Ges. **95,** 181–276 (1982).
[45] Wang, E., A. R. Goldberg: Binding of deoxyribonuclease I to actin: a new way to visualize microfilament bundels in nonmuscle cells. J. Histochem. Cytochem. **26,** 745–749 (1978).
[46] Whatley, J. M., F. R. Whatley: Evolutionary aspects of the eukaryotic cell and its organelles. In: A. Pirson, M. H. Zimmermann (eds.): Encyclopedia of Plant Physiology. Cellular Interactions. New Ser. Vol. 17. pp. 18–58. Springer-Verlag. Berlin, Heidelberg, New York, Tokyo 1984.