endogenous Ca^{++} to trigger histamine secretion from the mast cells cannot, however, be excluded. In this regard, it is noteworthy that some of the basic compounds like compound 48/80 and peptide 401 (the mast cell degranulating peptide from bee venom) give virtually optimal release in the absence of extracellular calcium[6].

The mechanism of D-galactosamine-induced histamine release from isolated rat peritoneal mast cells may be related to the mobilization of intracellular Ca^{++} from one or more of the calcium pools described by Pearce and his colleagues[12]. Further experiments using other divalent cations (e.g. Co^{++}, Mn^{++}) and organic calcium blockers to block calcium influx are suggested. These would help us to elucidate further the mechanism of action of D-galactosamine. D-galactosamine used in higher concentrations (7×10^{-3} M) failed to cause histamine release from the mast cells; this could be due to an induction of supra-optimal concentrations of Ca^{++} from the intracellular calcium pools which, in turn, inhibited histamine release. D-galactosamine is a derivative of 2-deoxy-D-galactose, which is a weak inhibitor of histamine release from mast cells[10]. It is therefore not surprising that at a higher concentration (7×10^{-3} M) DGM failed to release histamine from these cells. At this level, it could have acted as a metabolic poison in a way similar to that described for 2-deoxy-D-glucose[17,18].

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0014-4754/85/091177-02$1.50 + 0.20/0

Preferential destruction of chloroplast nucleoids in zygotes in green algae *Dictyosphaeria cavernosa* and *Acetabularia calyculus*

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Summary. The preferential destruction of chloroplast nucleoids in young zygotes in the coenocytic alga *Dictyosphaeria cavernosa* and the giant unicellular alga *Acetabularia calyculus* was studied by high resolution epifluorescent microscopy. The chloroplast nucleoids (DNA) in the chloroplast from one of the parents were preferentially destroyed soon after the mating of male and female gametes.
Key words. Preferential destruction; chloroplast nucleoids; *Dictyosphaeria cavernosa, Acetabularia calyculus.*

The unicellular isogamous green alga *Chlamydomonas reinhardtii* shows maternal transmittance of chloroplast genes like higher plants[1]. Therefore, the question whether or not uniparental transmittance of chloroplast genes or chloroplast DNA occurs in giant algae, which seem to be phylogenetically higher than *C. reinhardtii*, needs to be examined.

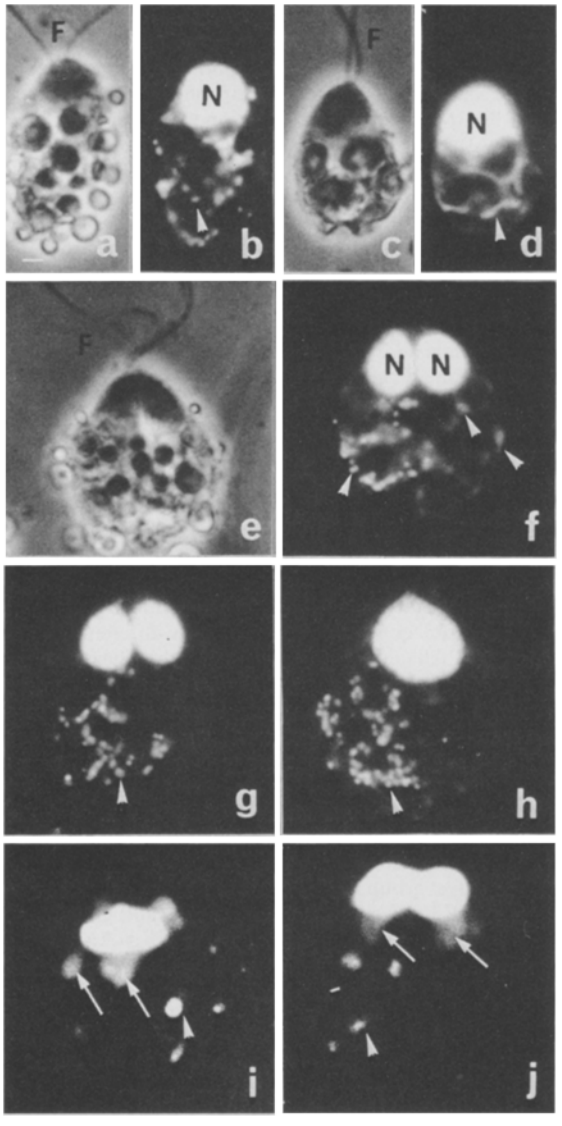
Previous studies on maternal inheritance in *C. reinhardtii* have shown by means of 4′,6-diamidino-2-phenylindole (DAPI) staining that the chloroplast nucleoids of male origin are destroyed shortly after mating, while the chloroplast nucleoids of female origin remain. Thus preferential destruction may account for the maternal inheritance of chloroplast genes[2]. DAPI staining is a simple and easy method for observing very small amounts of DNA in organelles[3–5].

The present experiments were done to observe the behavior of chloroplast nucleoids in young zygotes in green algae by means of DAPI staining.

Materials and methods. Dictyosphaeria cavernosa (Forssk) Børg was collected from the intertidal zone at Amami Island, Japan, placed in a separate glass vessel containing 100–150 ml of sterilized seawater and incubated according to a method described previously[6]. Swarmers of *D. cavernosa*, released about 1 month after initiation of the culture, were collected by utilizing their positive phototactic response. The sex of the gametes was determined by crossing tests. *Acetabularia calyculus* was originally collected from Wakasa Bay, Japan, and cultured autotrophically by methods described previously[7]. Cysts of *A. calyculus* were isolated from the caps and each cyst was placed in a separate watch glass. The gametes were released in 1–2 days after initiation of the culture. Female and male gametes of *D. cavernosa* and *A. calyculus* were mixed in a watch glass. The newly formed zygotes were fixed in 1% glutaraldehyde dissolved in buffer S[2] at 30, 60, 90 and 120 min after mixing of female and male gametes, and stored at 4 °C. They were stained with DNA fluorochrome DAPI and examined by Olympus BHS-RFK epifluorescent microscopy[2].

Results and discussion. Each gamete of *D. cavernosa* has two flagella about 15 μm long and contains a cell nucleus of 3 μm

diameter and a cup-shaped chloroplast (fig., a–d). Female gametes are yellowish green while male gametes are yellow[6]. After DAPI staining, each chloroplast emitting red fluorescence in the female gamete contained small spherical chloroplast nucleoids (fig., a, b). On the other hand, each chloroplast in male gametes contained small rod- and branch-like chloroplast nucleoids (fig., c, d) which emitted blue-white fluorescence. When gametes of different sexes were mixed, they aggregated immediately. After 5–10 min, about 60% of the gametes had fused. Their fusion began anteriorly, then gradually progressed laterally until the cells fused completely[6]. At 30 min after the male and female gametes had been mixed, the newly formed phanozygotes were heart-shaped or spherical, had four flagella and contained two discrete cell nuclei and chloroplasts (fig., e, f). The two cell nuclei were close together and the chloroplasts lay side by side at the base of the cell with the open ends of the cups facing the flagella. Chloroplasts of different parental origins had different-shaped chloroplast nucleoids soon after cell fusion had occurred (fig., f). At 60 min after mixing, when the cell nuclei of both gamete origins appeared to be touching each other, the branch- or rod-shaped chloroplast nucleoids from the male parent became faint (fig., g). When the cell nuclei fused to form a roundish nucleus, the chloroplast nucleoids of male gamete origin had disappeared completely, while the small spherical chloroplast nucleoids from the female parent remained (fig., g, h).



Phase-contrast (a, c, e) and epifluorescent (b, d, f–j) photomicrographs showing flagella (F), cell nuclei (N) and chloroplast nucleoids (arrowheads) in female gametes (a, b), male gametes (c, d) and zygotes (e–h) of *D. cavernosa* (a–h), and a gamete (i) and a zygote (j) of *A. calyculus* (i, j) at 30 min (e, f) and 60 min (g, h) after gametes of different sexes had been mixed. The cells were fixed in 1% glutaraldehyde for 30 s and stained with DAPI for 10 min, then squashed on a glass slide. a, c and e are phase-contrast images and b, d and f are epifluorescent images observed in the same field of view. At 60 min after mixing of male and female *D. cavernosa* gametes, the chloroplast nucleoids in the chloroplast from one of the parents had disappeared (right half in g, h), while those in the chloroplast from the other parent remained (left half in g, h). Similar preferential destruction of chloroplast nucleoids were observed in *A. calyculus* (right half in j). The preferential destruction of chloroplast nucleoids did not affect the cell nucleus and mitochondrial nucleoids of the same gamete origin and thus the mitochondrial nucleoids remained (arrows in j). Scale bar 1 μm.

Similar preferential destruction of chloroplast nucleoids was observed in young zygotes of *A. calyculus*. Each of the gametes of the alga was similar to that of *D. cavernosa* except that the mitochondrial nucleoids were so large that they could be seen under an epifluorescent microscope, and the chloroplast nucleoids of male gametes were not morphologically distinguishable from those of the female gametes. The cell nuclei of female and male gametes were spherical or football-shaped and contained dense chromatin which emitted a strong blue-white fluorescence. The chloroplast nucleoids showed a spherical or mesh-like structure embedded in the red fluorescence emitted from the chloroplasts. Large mitochondria were localized around the cell nucleus (8, fig., i) and the mitochondrial nucleoids had a lower fluorescent intensity than the chloroplasts and cell nuclei. Thus the sperical nucleoids in the mitochondria were easily distinguished from the cell nuclei and chloroplast nucleoids.

At 30 min after mixing of the gametes, the newly formed zygote had four flagella, two cell nuclei, several mitochondria and two chloroplasts. At 60 min after mixing, the chloroplast nucleoids in the cup-shaped chloroplast from one parent had disappeared completely while those from the other parent remained (fig., j). The cell nucleus and mitochondrial nucleoid of the same gamete origin also remained. These results suggested that the preferential destruction of chloroplast nucleoids does not affect the cell nucleus and mitochondrial nucleoids. Although it was difficult to determine whether or not the chloroplast nucleoids which disappear were of mt^- origin, it should be noted that the preferential destruction of chloroplast nucleoids of either chloroplast of both parent origins occurred. Similar changes have been observed during zygote formation in *C. reinhardtii*[2] and *C. moewusii*[9]. Since the preferential destruction of chloroplast DNA seems to be closely related to the maternal inheritance in *C. reinhardtii*, there must be maternal inheritance of chloroplast genes in these large algae and the preferential destruction of chloroplast DNA soon after mating may be a general phenomenon in isogamous green algae.

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