Acetabularia Chloroplast DNA: Electron Microscopic Visualization

Abstract. *Isolated chloroplasts of* Acetabularia mediterranea, *lysed by osmotic shock in a protein monolayer, released large amounts of supercoiled DNA. In some cases, the DNA radiated in large loops from centers of organization. The amount of DNA per chloroplast may be similar to that of a bacterium.*

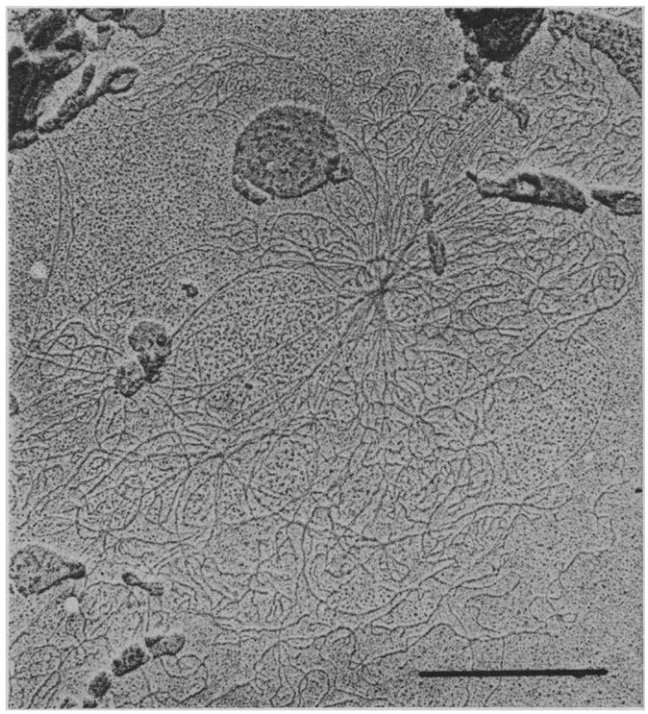
The amount of DNA in an organelle is an indication of how much autonomy it may have. We have studied the conformation and extent of DNA released from isolated chloroplasts of the green alga *Acetabularia mediterranea* to determine whether it is similar to that of a mitochondrion (*1*) or to that of a free-living bacterium (*2, 3*).

Acetabularia mediterranea was chosen because it is a very large (2 to 4 cm), uninucleate alga containing 10^6 to 10^7 chloroplasts per cell (*4*). The single nucleus is located in the rhizoid and can be removed by cutting off the base of the stalk. It is the only organism in which the possibility of nuclear contamination can be conclusively eliminated. In addition, its chloroplast DNA has been isolated chemically and determined to be a single species with a buoyant density of 1.704 g/cm^3 in CsCl (*5*).

The algae were cultivated (*6*) in an enriched seawater medium. They were not free of bacteria, but were washed frequently and treated with antibiotics before use. Chloroplasts were isolated in a solution of 0.4*M* sucrose, 0.02*M* tris(hydroxymethyl)aminomethane, 0.015*M* NaCl, and 0.01*M* ethylenediaminetetraacetate, *p*H 7.8, and purified by differential centrifugation and a discontinuous sucrose gradient (*5*). Electron microscopy of purified chloroplasts revealed about 1 mitochondrial profile in 200 and no bacteria. The chloroplasts, suspended in buffered sucrose, were diluted into denatured cytochrome c (1 mg/ml) in 2*M* ammonium acetate, and the suspension (20 to 40 μl) was spread on a distilled-water hypobase (*7*). The film, composed of DNA, chloroplast, and protein, was picked up on 200-mesh Formvar-coated grids, dried in air, and rotary-shadowed at 7° to 9° with platinum-palladium (80:20). This was followed by a second shadowing from a single direction or from two directions at right angles. Grids were examined with a Zeiss EM9A or a Hitachi HU-11A electron microscope. Only DNA attached to or in close proximity to chloroplast fragments was photographed.

A large amount of DNA was associated with chloroplast fragments. DNA released from a lysed chloroplast along with several thylakoids is shown in Fig. 1. A number of strands appear to radiate from a center. Even larger "displays" were released from some chloroplasts (Fig. 2). These had one or more centers of organization, similar to the "single-center" and "multicenter" displays released from *Hemophilus influenzae* (*2*). Although the DNA in Fig. 1 appears to be attached to the membranes in several places, most strands are just passing over or under membranes. However, we have occasionally seen a large number of strands radiating from what appears to be a rodlike extension of a lamella.

The DNA was frequently very highly supercoiled (Fig. 3). What appeared to be free ends at lower magnification were loops of DNA folding back on itself. The tightly supercoiled regions have diameters of about 135 Å; those of linear (untwisted) regions have diameters of 70 to 90 Å—that is, within the range of ordinary duplex DNA (*7*). Supercoiling does not, however, imply



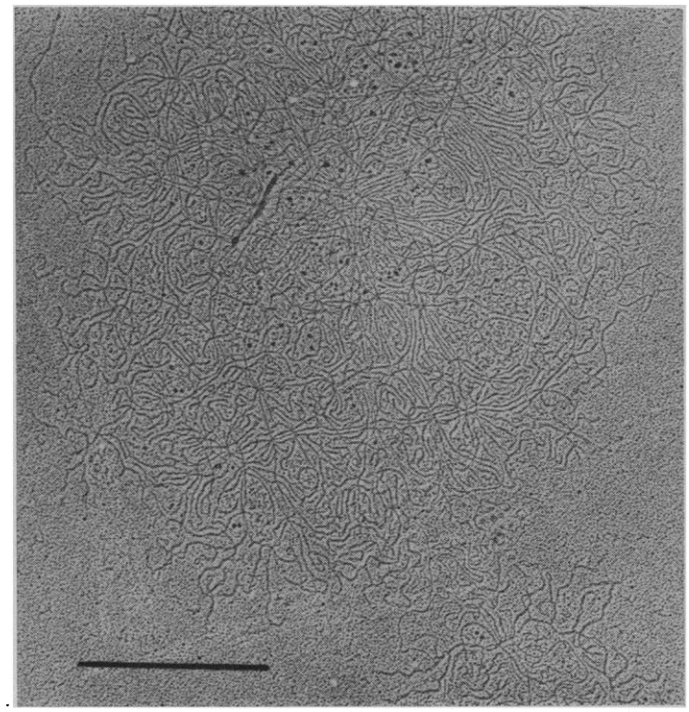


Fig. 1 (left). Lysed chloroplast of *A. mediterranea* with DNA fibrils around a "center"; Pt-Pd shadowed. Note particles on surface of isolated thylakoid. The bar represents 1 μm. Fig. 2 (right). Large "display" of chloroplast DNA. The bar represents 1 μm.

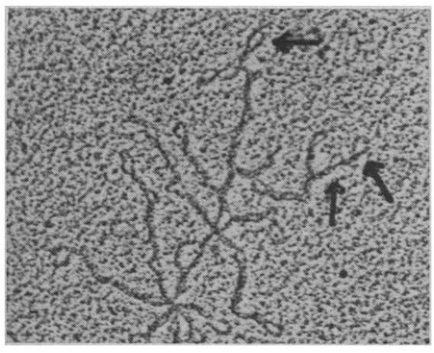


Fig. 3. Highly supercoiled chloroplast DNA. Arrows point to loops (about × 75,000).

that the DNA is circular, as it could be the result of replicative unwinding (*8*). Although few or no free ends could be seen, it was impossible to say that the tangled mass of a display consisted of only one molecule.

Acetabularia chloroplasts did not release anything resembling the single-stranded "mesh" DNA of spinach chloroplasts (*9*). Although strands about 15 Å in diameter were seen in some displays, they were always near other strands or stroma debris, whose effect on width of rotary-shadowed DNA is unknown. We did not see anything resembling the "hybrid loops" reported for *A. crenulata* chloroplast DNA (*10*).

Between 20 and 40 percent of the ruptured chloroplasts had DNA associated with them. The percentage depended on the degree of intactness of the chloroplast preparation. This is probably due to the presence of an intrachloroplastal nuclease, because chloroplasts incubated in buffered sucrose at 37°C for half an hour before spreading released only short fragments. The addition of deoxyribonuclease just before spreading produced the same effect. In addition, some of the DNA could be lost into the hypophase.

Attempts to isolate intact molecules by using phenol extraction resulted in extensive breakage. The largest amount of DNA released by osmotic shock which was untwisted enough to measure was 419 μm in length, corresponding to a molecular weight of 805×10^6 daltons (*11*). This suggests that the amount of DNA per chloroplast is in the same range as that of bacteria (*2, 3*) and within the range of DNA content determined chemically for chloroplasts of *Chlorella* (*12*), *Chlamydomonas* (*13*), *Euglena* (*14*), and lettuce (*15*). This amount is much greater than that of a mitochondrion (*1*). Whether the chloroplast DNA consists of unique sequences or highly redundant ones (that is, its information content) will have to be determined by renaturation kinetics (*15*).

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Myxovirus Antibody Increases in Human Connective Tissue Disease

Abstract. *Antibodies to measles and parainfluenza type 1 viruses were significantly increased in systemic lupus erythematosus and Reiter's syndrome. Of the individuals with highest titers of measles antibody, 75 percent had neurologic illness. Persistent virus infection may be a factor in the pathogenesis of these diseases.*

The human connective tissue diseases are characterized by abnormal immunologic processes, which appear, in part, to mediate pathogenesis. However, the stimulus initiating this abnormal response, and hence the etiology of these diseases, remains obscure. There has been renewed interest in the role of microbial agents as initial, and perhaps persisting, etiologic factors (*1*). A possible parallel exists in the evolution of knowledge about subacute sclerosing panencephalitis (SSPE), where initially myxovirus-like inclusions were found in brain tissue by electron microscopy, next the titers of serum antibodies to measles were shown to be increased, and then measles virus was isolated from brain (*2*). In view of these findings, the discovery of similar microtubular inclusions in renal tissue from a patient with systemic lupus erythematosus (SLE) was of interest (*3*). These morphologic observations have been confirmed and extended, but their real nature and specificity is unclear (*4*). Measles and the other viruses known to cause such inclusions belong to the paramyxovirus group. We now report on the concentration of antibodies to two of these viruses in patients with connective tissue diseases.

Patients were divided into groups according to their clinical diagnoses. The SLE group was further subdivided into those with (SLE + N) and those without (SLE − N) neurologic symptoms or signs, since an earlier study suggested that the titer of antibodies to measles was higher in the former (*5*). Of the six patients with Reiter's syndrome, four had all three typical characteristics (arthritis, urethritis, and conjunctivitis). The rheumatoid arthritis group contained 15 patients with classical or definite, and five with probable, rheumatoid arthritis. The juvenile rheumatoid arthritis group contained three patients with definite, and four with possible, disease. The other connective tissue disease group had six with progressive systemic sclerosis, five with vasculitis, two each with dermatomyositis, "overlap" syndrome (features of several distinct connective tissue diseases), and Dilantin-induced SLE, and one with arthritis and hypogammaglobulinemia. The miscellaneous group included ten patients with arthritides other than rheumatoid arthritis, eight members of two families in each of which several children had polyarthritis of undetermined etiology, four patients with undiagnosed neurologic illness, and ten with various other conditions. Serums were obtained from three patients with SSPE (*6*). The normal group was drawn from physicians and laboratory personnel. Serums were stored at −20°C. Subsequent analysis