Acta Medica Okayama

Volume 21, *Issue* 1 1967 *Article* 1

FEBRUARY 1967

Effect of DNase on DNA-like fibrils in chloroplasts and mitochondria

E-iti Yokomura*

*Okayama University,

Effect of DNase on DNA-like fibrils in chloroplasts and mitochondria*

E-iti Yokomura

Abstract

For the purpose to confirm the localization of DNA in chloroplasts and mitochondria, the cells of Spinacia oleracea fixed with glutaraldehyde-Os04 were observed by electron microscope with or without DNase treatment. "DNA fibril complexes" have always been found in the electron-transparent regions of the chloroplasts and mitochondria of the cells receiving no DNase treatment. By treating with DNase, the DNA fibril complexes of these organellae are reduced considerably in their density, leaving only faintly visible ghostlike structure or having completely disappeared. These observations confirm that the DNA fibril complexes in chloroplasts and mitochondria as demonstrated by glutaraldehyde-OsO4 fixation are the DNAcontaining structures similar to those found by formalin or buffered OsO4 fixation, and suggest that it will have only a small amount of the material other than DNA distinct from the case of DNA in the nucleus.

*PMID: 4229053 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 21, 1~7 (1967)

EFFECT OF DNASE ON DNA-LIKE FIBRILS IN CHLOROPLASTS AND MITOCHONDRIA

E-iti YOKOMURA

Department of Pathology, Okayama University Medical School, Okayama, Japan (Director: Prof. S. Seno)

Received for publication, December 5, 1966

RIS and PLAUT (1962)[1] have found microfibrils in the chloroplasts of *Chlamydomonas moewusii*, which appeared in the sections of the cell in electron microscope. These microfibrils disappeared by the treatment of the formalin-fixed tissue with DNase, and they concluded that the fibrils correspond to the DNA macromolecules. In 1963 NASS and NASS[2] also found fibrous structures in the mitochondria of chick embryo cells, revealing that the structures were obliterated by DNase digesion. KISLEV and others (1965)[3] demonstrated similar structures in the chloroplasts and mitochondria of *Beta vulgaris*, which also proved to be the DNA containing structure through the digestion test with DNase. YOKOMURA[4,5] observed chloroplasts and mitochondria of the cells from lower to higher plants of eight phyla, and revealed the existence of the "DNA fibril complexes" in chloroplasts and mitochondria of all the plants so far he observed. This fact indicates that the "DNA fibril complexes" should be the component common to chloroplasts and mitochondria of the plant cells as in the case of mitochondria of animal cells, though the morphologic patterns differed somewhat from species to species.

This paper deals with the digestion-test of "DNA fibril complexes" with DNase, which were demonstrated by glutaraldehyde-OsO_4 fixation of the chloroplasts and mitochondria of *Spinacia oleracea*, and the precise structures of the fibrillar element seen after the digestion by DNase.

MATERIALS AND METHODS

Small pieces of young leaves of *Spinacia oleracea* were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer for two hours in cold, then washed with the cacodylate buffer overnight in cold. After washing the samples were sectioned into very small pieces with hand and immersed in 0.05 M acetate-veronal buffer containing 0.003 M $MgSO_4$ (pH 6.4). Then the samples were divided into two groups, one for light microscopy and the other for electron microscopy.

For the light microscopy, the samples were transferred to the acetate-veronal buffer containing DNase (Sigma, crystalline, 1 mg/ml of the buffer) (NASS and NASS[2], 1963) and incubated at 37°C for 8, 12, 16 and 24 hours. Control samples were incubated at 37°C with the buffer solution containing no DNase for the same durations as in the experimental series.

After incubation all the samples were washed with the veronal-acetate buffer several times, then washed with water, dehydrated through ethanol series and embedded in Epon 812. Sections were cut to 2 μ thick, mounted on glass slides and stained with Feulgen reaction by a method of RIS and PLAUT[1] (1962), *i. e.*, the sections were hydrated in N HCl at 60°C for 20 minutes and stained with Schiff's reagent for one hour. For electron microscopy the samples were incubated in the acetate-veronal buffer containing DNase (Sigma, crystalline, 1 mg/ml of the buffer) and no DNase for control at 37°C for 16 and 24 hours. After incubation all the samples were washed with the acetate-veronal buffer several times, transferred to the cacodylate buffer, postfixed with 1% OsO_4 in the cacodylate buffer, dehydrated through ethanol series and embedded in Epon 812. Thin sections were cut on a Porter-Blum MT-1 microtome, stained with uranyl acetate and/or lead hydroxide (KARNOVSKY[6], 1961), and observed in an electron microscope, Hitachi HU-11 A.

RESULTS

Light microscopic observation of the cells stained by Feulgen reaction revealed that DNA was digested completely by exposing the cells to the 0.1% DNase solution in acetate-veronal buffer for 16 and 24 hours at 37°C, while in the controls Feulgen reaction stained clearly the nuclei of the cells in the tissue sections. Incubation of the tissues with the medium having no DNase did not change the staining intensity of the nuclei by Feulgen reaction. Eight-hour incubation with the medium containing DNase at 37°C resulted in a slightly reduced intensity of the staining, 12-hour incubation induced a marked reduction in the reaction intensity and a completely negative reaction after 16- and 24-hour incubation. In any case the cytoplasm gave always negative reaction. The result indicates that the DNA is completely digested by exposing the tissue sections to the DNase solution for 16 to 24 hours.

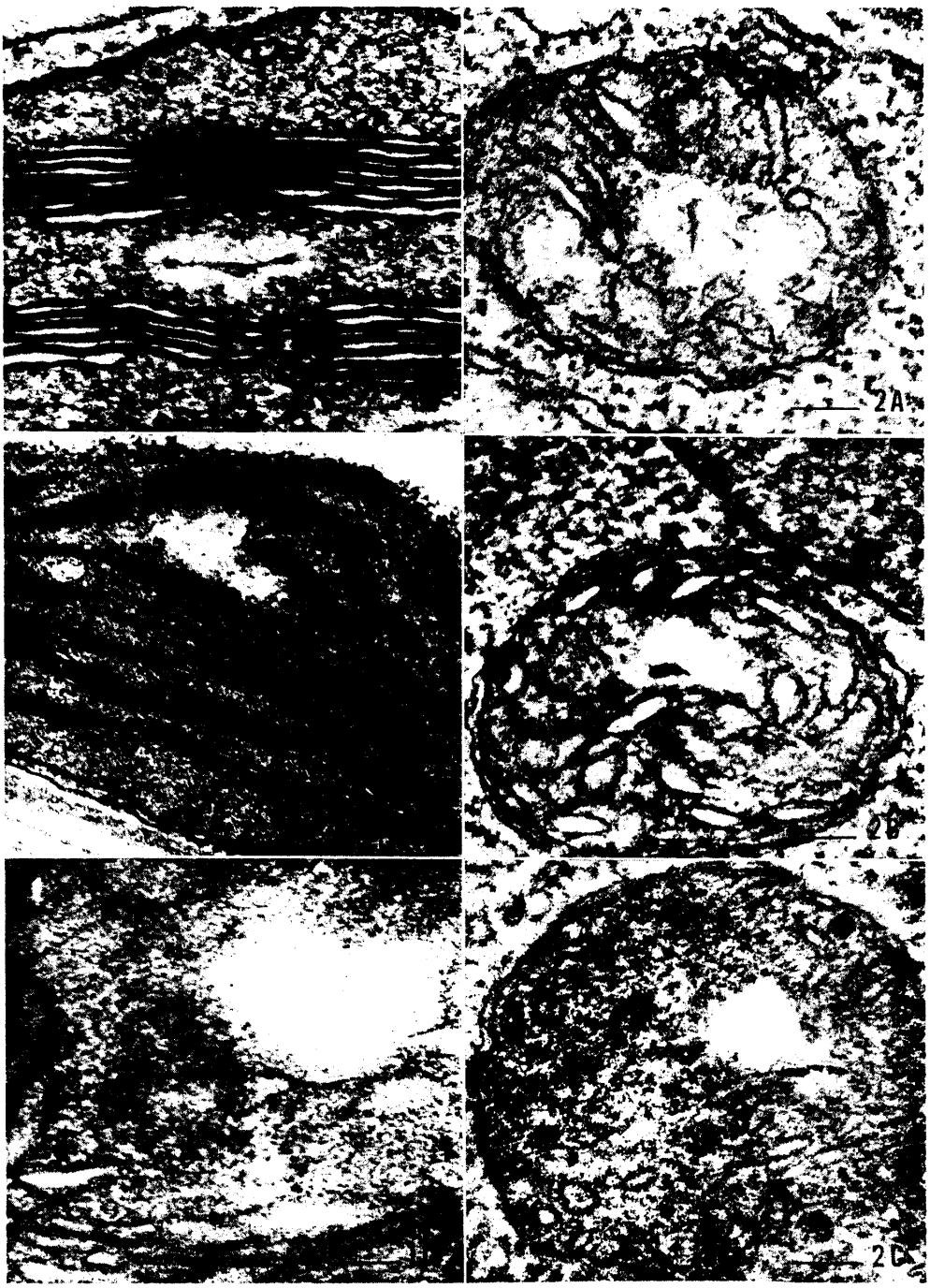
On the basis of these light microscopic observations, electron microscopic observations on the DNA fibril complexes of chloroplasts and mitochondria have been carried out with or without digestion test of DNA by DNase. As reported in the previous papers (YOKOMURA[4,5]), the present study has also revealed the same structures in the cells of *Spinacia oleracea* fixed with glutaraldehyde-OsO_4, that is, the DNA fibril complexes appeared as fibrous structures in the

electron-transparent regions of matrixes of both chloroplasts and mitochondria. In the control series of experiments, receiving no DNase treatment, the DNA fibril complexes were found both in the chloroplasts and mitochondria of *Spinacia oleracea.*

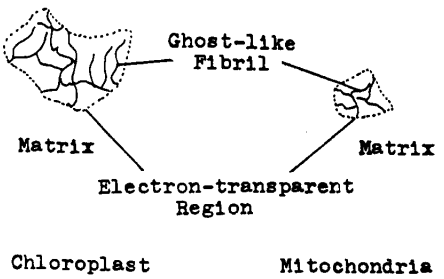
As can be seen in Figs. 1-A and 2-A the DNA fibril complexes of this species have core-like center in the fine fibrillar structures. Sixteen-hour incubation of the tissues at 37°C with the acetate-veronal buffer containing no DNase does not induce any actual change in the morphologic appearance of the DNA fibril complexes. They are observed in the transparent regions of chloroplasts and mitochondria as in the non-incubated cells (Figs. 1B and 2B respectively). However, the incubation with DNase containing buffer at 37°C for 16 hours alters considerably the morphologic appearance of the DNA fibril complexes. In some cells their chloroplasts and mitochondria have lost the DNA fibril complexes leaving hardly visible residual structures, but some others still show the fibrous structures in the electron-transparent regions of both organellae. After DNase treatment, these fibrous structures appear less electron-dense comparing to those of control, but the ghosts of the fibrous structures are still visible. The exposure to DNase for 24 hours effects no changes as compared with those exposed for 16 hours, indicating that the DNA fibril complexes are composed of DNA and some small amount of the material other than DNA. The effect of DNase on both organellae, chloroplasts and mitochondria, is the same. The other structures of both organellae are not affected by the incubation with DNase. The morphologic structures of nuclei are actually not altered even after the treatment with DNase for 16 and 24 hours, though dense granules and fibrils observed in karyolymph grow less dense than in the controls. Similar observations on nuclei after the treatment with DNase have been reported by RIS and PLAUT[1] (1962) and NASS and NASS[2] (1963).

DISCUSSION AND CONCLUSION

As has been briefly described, the recent morphologic studies of chloroplasts and mitochondria have revealed DNA containing structures in these organellae. Moreover, the recent biochemical assays have also confirmed the presence of DNA in chloroplasts and mitochondria (SAGER and ISHIDA[7], 1963; LUCK and REICH[8], 1964), and it has been demonstrated that these organellae have the specific DNA different from that of nuclei, suggesting that the choroplasts and mitochondria are the self-duplicating organellae. However, it is left unclarified whether or not these DNA containing structures are the components of these organellae that are invariably found in all species of plants from lower to higher ones. Previous works of the author (YOKOMURA[4,5]) have suggested that the DNA


2A
2B
2C

fibril complexes are the components common to these two organellae, and the present study has revealed the DNA fibril complexes, which can be found by glutaraldehyde-fixation and post-osmication, are the DNA containing structures similar to those observed in choroplasts and mitochondria of some plants by the formalin fixation (RIS and PLAUT[1], 1962; KISLEV *et al*[8], 1965).



Figs. 1D and 2D Schemes of ghost-like fibrils which are faintly visible in Figs. 1C and 2C respectively.

In the present study cytoplasmic staining gave Feulgen negative reaction. However, this result may not necessarily mean the absence of DNA in chloroplasts and mitochondria. The dimensions of the DNA fibril complexes, which are regarded to contain DNA in both organellae, are generally less than 0.1 μ in diameter and below the resolution of light microscope. Supposing the DNA

The Bar in Each Photograph is in the Unit of 0.1 μ.

Figs. 1A~1C "DNA fibril complexes" of chloroplasts of *Spinacia oleracea*.

Fig. 1A "DNA fibril complex" seen in the chloroplast after glutaraldehyde-OsO_4 fixation. An elongated core-like structure in association with fine fibrils can be seen in the electron-transparent region. No DNase treatment. ×100, 000

Fig. 1B the similar structure as in Fig. 1A but incubated with acetate-veronal buffer containing 0.003 M $MgSO_4$ at 37°C for 16 hours. The matrix of chloroplast appears dense but the "DNA fibril complex" is seen in the electron-transparent region. ×50, 000

Fig. 1C the picture of the electron-transparent region after the treatment with DNase at 37°C for 16 hours. The electron-dense picture of "DNA fibril complex" has disappeared but some fibrils may be seen persisting through DNase digestion. ×100, 000

Figs. 2A~2C "DNA fibril complexes" of the mitochondria.

Fig. 2A "DNA fibril complex" having some core-like structure in the central area of the fibrils. Glutaraldehyde-OsO_4 fixation, no treatment with DNase. ×100, 000

Fig. 2B morphologic pattern of "DNA fibril complex" after the incubation with the acetate-veronal buffer at 37°C for 16 hours. No DNase treatment. The core-like structure is clearly visible. ×100, 000

Fig. 2C the transparent region of a mitochondrion treated with DNase at 37°C for 16 hours. The picture of the "DNA fibril complex" has disappeard leaving only scarcely visible fibrous structures. ×100, 000

fibril complexes to be Feulgen-positive, then these should not be detected by light microscopy. The problem is whether or not the DNA fibril ccmplex has some structural protein, as in the case of chromonema of the nucleus. Electron microscopic observations of the nuclei after the treatment with DNase, where the nucleus gives completely negative Feulgen reaction, have revealed distinct structures in nuclei whose morphologic pattern is nearly the same as those seen without any DNase treatment. RIS and PLAUT[1] (1962), NASS and NASS[2] (1963) and KISLEV *et al.*[3] (1965) reported that the DNA containing stucture of chloroplasts and mitochondria are completely obliterated by DNase treatment. This may mean that the DNA fibril complex is just the DNA macromolecule itself and has no detectable structural protein. The present study on the chloroplasts and mitochondria of *Spinacia oleracea* has reconfirmed the observations made by several authors just mentioned, revealing that some organellae have lost completely the DNA fibril complex after digesting with DNase. However, in some cases there remains a ghost-like structure after DNase treatment, though it is scarcely visible in some cases. Longer incubation with DNase has never altered this picture. This fact seems to suggest that the DNA fibril complexes of chloroplasts and mitochondria will actually be of DNA with some very small amount of associated material. Therefore, if chloroplasts and mitochondria could replicate with their DNA, the mechanism of replication would be different from the case of nucleus.

SUMMARY

For the purpose to confirm the localization of DNA in chloroplasts and mitochondria, the cells of *Spinacia oleracea* fixed with glutaraldehyde-OsO_4 were observed by electron microscope with or without DNase treatment.

"DNA fibril complexes" have always been found in the electron-transparent regions of the chloroplasts and mitochondria of the cells receiving no DNase treatment. By treating with DNase, the DNA fibril complexes of these organellae are reduced considerably in their density, leaving only faintly visible ghost-like structure or having completely disappeared.

These observations confirm that the DNA fibril complexes in chloroplasts and mitochondria as demonstrated by glutaraldehyde-OsO_4 fixation are the DNA-containing structures similar to those found by formalin or buffered OsO_4 fixation, and suggest that it will have only a small amount of the material other than DNA distinct from the case of DNA in the nucleus.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Professor N. SHINKE of Kyoto University and Professor S. SENO for their invaluable advice and suggestion for this investigation.

REFERENCES

1. RIS, H. and PLAUT, W.: Ultrastructure of DNA-containing areas in the chloroplast of *Chlamydomonas*. *J. Cell Biol.* 13, 383～391, 1962
2. NASS, S. and NASS, M. M. K.: Intramitochondrial fibers with DNA characteristics II. Enzymatic and other hydrolytic treatments. *J. Cell Biol.* 19, 613～629, 1963
3. KISLEV, N., SWIFT, H. and BOGORAD. L.: Nucleic acids of chloroplasts and mitochondria in Swiss chard. *J. Cell Biol.* 25, 327～344, 1965
4. YOKOMURA, E.: An electron microscopic study of DNA-like fibrils in chloroplants, *Cytologia*, in press, 1967 a
5. Yokomura, E.: An electron microscopic study of DNA-like fibrils in plant mitochondria, *Cytologia*, in press, 1967 b
6. KARNOVSKY, M. J.: Simple methods for "staining with lead" at high pH in electron microscopy. *J. biophysic. biochem. Cytol.* 11, 729～732. 1961
7. SAGER, R, and ISHIDA, M. R.: Chloroplast DNA in *Chlamydomonas*. *Proc. nat. Acad. Sci. U. S.* 50, 725～730, 1963
8. LUCK, D. J. L. and REICH, E.: DNA in mitochondria of *Neurospora crassa*. *Proc. nat. Acad. Sci. U. S.* 52, 931～938, 1964