Multiple Amounts of DNA Related to the Size of Chloroplasts

II. Comparison of Electron-microscopic and Autoradiographic Data

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Summary

Complete section series of young protease-treated plastids have confirmed our previously reported data on autoradiographic experiments. The DNA of one plastid can be localized in several distinct regions. The amount of DNA is related to the size of the plastid.

Chloroplasts contain DNA. However, even for the more simply constructed chloroplasts of higher plants there is almost no exact information as to their spatial distribution in the stroma, the amount per organelle and the variability of this quantity.

Autoradiographic examination of an experimentally favourable material (Beta vulgaris L.; material, methods, and figures see Herrmann 1970) produced the following results: (1) Counts of silver grains revealed a clear dependency (the scatter diagram, if plotted logarithmically, shows that the bivariate distribution forms a rather narrow ellipse) of the incorporated $^3$H-thymidine quantity per organelle on the size of the latter. This is true if it is assumed that chloroplasts have approximately the same thickness (and density) independently of their size (Figs. 2 and 3), and is also true of chloroplasts from plants with plastids of genetically different size and from leaves of one clone in different developmental stages. (2) The presence of several DNA-regions in chloroplasts could be deduced from the number of accumulations of silver grains above the organelle. These labelled centres appear circular above young plastids and above older chloroplasts elongated, sometimes branched, and usually some distance apart. The number of the centres
and the size of the organelle are correlated (Fig. 1). This result could be explained by a polyenergid organization of the chloroplast. The autoradiographic method does not allow more precise statements because (1) centres situated above each other cannot be detected, (2) vice versa the two ends of a figure-eight-shaped centre can be counted separately, and (3) in very small plastids (< 3 μ) the question of the number of centres cannot be answered due to the resolution of 1 μ, which is the best that can be achieved. An observed relatively large number of silver grains above small plastids could be explained either by a larger amount of DNA, by reduced absorption of electrons due to a matrix of lower density, or by changed rates of incorporation in small plastids.

In complete section-series of chloroplasts the distribution of DNA should thus be presented electron-microscopically and therefore directly. In contrast to mitochondria, of which the size often does not exceed 1.5 μ and which contain only 1–2 clearly demonstrable nucleoids, the greater volume of the chloroplasts presents considerable difficulties during preparation (with a section-thickness of 700 Å about 70 individual sections would be necessary in order to cut a chloroplast of 5 μ completely). It is, however, much more difficult to follow the spatial distribution of DNA-regions within the ribosome-containing dense matrix. We succeeded in overcoming these difficulties by almost complete digestion of the matrix and in exposing the DNA-containing regions while preserving the ultrastructure (method see Herrmann and Kowallik 1970). The autoradiographic results as to DNA-distribution in small chloroplasts are now completed and confirmed by several section-series (3 of them complete) of various large chloroplasts from embryonic leaf-tissue.

The series were evaluated from two points of view: (1) after planimetric measurements of the areas occupied by the chloroplast and the DNA-containing structures the volumes of plastids and DNA-areas were calculated by means of the formula for the spherical segment (ends of chloroplasts), for the cone (ends of DNA-regions) and for the obtuse cone, based on a mean section-thickness of 700 and 1000 Å. (2) Models of the chloroplasts and DNA-areas were reconstructed out of slices corresponding to the individual sections (Figs. 4, 4*; 5, 5*; 6). The results are summarized in Table 1.

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1 The term "Polyenergide" was originally coined by Sachs (1892) for multinucleate eucaryotic cells, but was later applied to blue-green algae (cf. Geitler 1964).

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Fig. 1. Number of labelled centres (in %) related to the size of chloroplasts (2/2, 3/3 etc. diameters of plastids in μ; n = number of chloroplasts, which could be evaluated). Left: Chloroplasts of progeny 1138. Euploid (small plastids) and trisomic (large plastids) plant distinguished by different colours. Tissue samples incubated for 18 hrs in a solution containing H-thymidine. Right: Chloroplasts of progeny 23. Both plants and all label times are included. Isolation, treatment, and Figs. of chloroplasts see Herrmann (1970)