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VARIABLE AMOUNTS OF DNA RELATED TO THE SIZE OF CHLOROPLASTS

IV. THREE-DIMENSIONAL ARRANGEMENT OF DNA IN FULLY DIFFERENTIATED CHLOROPLASTS OF *BETA VULGARIS* L.

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SUMMARY

Glutaraldehyde-fixed strips of fully differentiated leaves of *Beta vulgaris* L. were treated with proteases. With this method it is possible to display DNA-regions in chloroplasts selectively and to reconstruct them from serial sections.

As in young plastids, the DNA in mature plastids is also distributed within several regions which are separated rather clearly by thylakoids. The *number* of such regions depends upon the size of the organelle and thus upon its developmental state. The *shape* of the regions is, in contrast to less-differentiated plastids, mostly elongated. The individual regions seem to contain unequal quantities of DNA-fibrils.

A comparison of our ultrastructural results and biochemical data (DNA-amounts per plastid depending on the size of the organelle; kinetic complexity of chloroplast-DNA of *Beta*) as well as a comparison of the chloroplast with similar prokaryotic systems (as in bacteria, blue-green algae, and mitochondria) leads to the suggestion that each DNA-containing region can be regarded as a single nucleoid. In addition, each nucleoid already contains several (on average, 4–8) genetic units. Thus the chloroplast of *Beta* seems genetically polyvalent in at least 2 respects: (i) it is of *polyenergidic* organization, and (ii) the individual nucleoids can be *polyploid* to varying degrees.

INTRODUCTION

Cytological and quantitative biochemical data on chloroplast-DNA have not yet been combined in one plant species. The following 3 main aspects have been considered separately from each other in former publications: (i) How much genetic information does the chloroplast genome contain (Wells & Birnstiel, 1969; Herrmann, 1970*a*; Tewari & Wildman, 1970; Bastia, Chiang, Swift & Siersma, 1971; Wells & Sager, 1971)? (ii) What is the DNA-content of one chloroplast (for review, see Smillie & Scott, 1969)? How many chloroplast genomes correspond to this amount (Wells & Birnstiel, 1969; Bastia *et al.* 1971)? How varied is the number of genomes per chloroplast (Herrmann, 1970*a*, 1971; Bastia *et al.* 1971)? (iii) How is the DNA arranged within the chloroplast (Bisalputra & Bisalputra, 1969; Herrmann & Kowallik, 1970*b*; Kowallik & Haberkorn, 1971)? We have tried to consider the entire problem with different techniques in *one* object (*Beta vulgaris* L.).

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Light-microscopic autoradiography provided evidence of the arrangement of DNA within the chloroplast and of a variation of the DNA-content per organelle (Herrmann, 1970*b*). It was suggested that the DNA is distributed within several regions; the quantity of DNA as well as the number of DNA-regions and the size of the plastids seemed to be correlated. Because of the limitation of the autoradiographic method it was necessary to check both the quantitative aspect with biochemical techniques (Herrmann, 1972) and the cytological aspect using electron microscopy (Herrmann & Kowallik, 1970*b*). In a continuation of the series of papers (Herrmann, 1970*b*; Herrmann & Kowallik, 1970*b*; Herrmann, 1972) the present report is meant to answer the questions concerning the 3-dimensional arrangement of DNA in fully differentiated chloroplasts by electron microscopy, and to arrive at a synthesis of those results which have been obtained from the investigations mentioned above.

Spatial reconstruction of DNA-regions from serial sections is, however, often prevented due to the electron-opaque chloroplast matrix. Only after proteolytic digestion of the chloroplast matrix was it possible to obtain a sufficiently selective presentation of DNA-regions without destroying the thylakoid system (Herrmann & Kowallik, 1970*a*). With this method it was possible to confirm that the DNA within the organelle is distributed over several matrix regions. The single regions are rather clearly separated by thylakoids. This was shown chiefly by analysing complete series of sections of plastids with different but relatively small diameters (1·1–2·8 μm). Even these small plastids contain several (up to 5) such regions (Herrmann & Kowallik, 1970*b*).

A comparable fine-structural analysis of fully differentiated chloroplasts of higher plants has yet to be performed. According to our autoradiographs, the number, size and shape of these regions should change during the development of the organelle (Herrmann, 1970*b*). At present the protease digestion seems to us to be the only way to make relatively exact statements on the 3-dimensional arrangement of DNA, even in old chloroplasts, although it causes some structural changes in these chloroplasts as compared with proplastids and etioplasts. However, these structural alterations, which concern mainly the fragmentation of the chloroplast envelope, are virtually irrelevant to our problem.

MATERIALS AND METHODS

The material, trisomic type VIII of *Beta vulgaris* L. (Butterfass, 1967) and its eudiploid control, was the same as that used for the previous investigations (Herrmann, 1970*a*, *b*; Herrmann & Kowallik, 1970*a*, *b*). Trisomic plants of type VIII contain chloroplasts which differ markedly in size from those of eudiploid sister plants (see figs. 1 and 2 in Herrmann, 1969).

Small strips of fully developed leaves, the chloroplasts of which measured up to 15 μm in diameter, were fixed in 4% glutaraldehyde made up in 0·05 M cacodylate buffer, pH 7·0, for 6 h. After several rinsings in Tris buffer, the material was treated with proteases (trypsin or Pronase; for discussion of the method, see Herrmann & Kowallik, 1970*a*). It is sufficient to say that protease treatment for 8–16 h results in a nearly complete dissolution of the matrix of organelles and the ground plasm, including ribosomes. Membranes except nuclear and chloroplast envelope exhibit no visible attack by the enzyme; even the 3-layered unit membrane structure of thylakoid membranes can be distinctly resolved after protease treatment (cf. also Kowallik & Haberkorn, 1971).

Post-osmication, dehydration and embedding in Epon 812 were by standard procedures. Serial sections were prepared on the LKB Ultrotome III, mounted on carbon-coated single-

hole or slot grids, double stained with uranyl acetate and lead citrate and examined in a Siemens electron microscope Elmiskop I A.

Depending on the degree of protease digestion, the DNA in chloroplasts appears either fluffy or fibrillar (Figs. 4, 5). The fluffy aspect which results from incomplete tryptic digestion is presumably caused by digestion products of matrix proteins which become electrostatically linked to the DNA-fibrils. It was noticed that in small plastids the DNA seems to be homogeneously arranged within distinct matrix regions (Herrmann & Kowallik, 1970*a*), thus allowing the DNA-containing regions to be reconstructed from serial sections and analysed volumetrically. However, the assumption has to be made that either the protease treatment does not cause an essential alteration of the size of the DNA-regions, or the alterations in size of the individual regions, if they really occurred, would be of the same degree of order.

The increased transparency of the sections after trypsin treatment permitted use of a maximum section thickness which was estimated as approximately 100 nm from the interference colours of the ribbon floating on water. Thus it was possible to cut relatively large parts of fully developed chloroplasts, although complete serial sections of whole chloroplasts were not obtained. It proved impossible, however, to analyse most of the DNA-regions of mature chloroplasts with the methods used for differentiating plastids (Herrmann & Kowallik, 1970*b*), since the DNA in mature chloroplasts seems to be arranged rather inhomogeneously within the interthylakoid regions. In addition, if digestion of the organelle matrix was incomplete, it appeared difficult or even impossible to make a clear distinction between small DNA-containing structures and fluffy components of incompletely digested matrix material (Fig. 2).

RESULTS

The electron micrographs of serially sectioned chloroplasts of fully differentiated leaves confirm our conclusions from the autoradiographs in all details: as in differentiating plastids (Herrmann & Kowallik, 1970*b*), the DNA in fully grown chloroplasts is also distributed within several regions, abbreviated as 'DNA-regions' in the following (Table 1, Figs. 2–6).

Shape of DNA-containing structures

The DNA-containing structures in less-differentiated plastids (Fig. 1) are generally more compact than those in chloroplasts with full photosynthetic activity (Figs. 2–4, 6, but see also Fig. 5) and mostly exhibit a disk-like shape (Herrmann, 1970*b*; Herrmann & Kowallik, 1970*b*). According to the more flattened or elongated matrix regions, the DNA-containing structures of fully developed chloroplasts are usually stretched. This can be observed especially in those serial sections where the plane of the section is parallel to the direction of the thylakoid membranes, which are thus in surface view (Figs. 6, 9). Depending on the incubation time, the DNA-structures appear either with a distinct outline or more or less disintegrated so that individual DNA-fibres become virtually attached to adjacent thylakoids (Figs. 3, 4, 8).

One might expect from the autoradiographs that the DNA-regions in fully differentiated chloroplasts are ***branched*** (even in less differentiated chloroplasts some indications of branching DNA-regions were evident, cf. Herrmann & Kowallik, 1970*b*). This seems true, although less frequently than assumed originally, since in autoradiographs superimposed DNA-regions may cause an erroneous impression of branching because of the lack of spatial resolution (Fig. 7). In our serial sections we also observed transition stages between apparent connexions of neighbouring DNA-regions caused by individual DNA-strands and entirely separate DNA-structures.

Table 1. *Volumes of chloroplasts (Cp) and numbers of their DNA-regions as reconstructed from serial sections*

Cp volume,† μm^3	No. of DNA-regions*	Cp maximal		Part of the serially sectioned Cp, %‡	Plant type	Fig. no.
		Length,† μm	Height, μm			
4·8	*4*	2·4	1·4	100	E	1
18	*7*	5·3	1·2	*ca.* 80	T	6, 9
21	*4–5*	5·6	1·3	45	E	—
22	*5*	5·9	1·2	20	T	—
24	*8*	5·5	1·5	50	T	—
26	*7–8*	6·0	1·4	52	T	—
26	*9*	6·5	1·2	30	T	—
28	*7*	6·2	1·4	45	T	4
29	*3*	5·7	1·7	25	T	—
34	*4*	6·2	1·7	30	T	—
34	*7*	7·4	1·4	30	T	—
38	*8–9*	7·0	1·5	30	T	3, 8
42	*18*	6·9	1·7	25	E	5
43	*11*	5·6	2·6	30	E	—
44	*11*	6·3	2·1	30	E	—
49	*9–10*	8·5	1·3	35	T	—
56	*7*	8·2	1·6	35	E	2, 7
67	*9*	7·8	2·1	37	E	—
69	*9*	7·4	2·4	25	E	—
84	*9*	8·2	2·4	35	E	—
98	*12*	10·5	1·7	20	T	—
114	*10*	11·3	1·7	20	T	—

* For the difficulties involved in arriving at a definite number of DNA regions, see text (Results). The numbers of DNA regions are minimal values, since only a part of each chloroplast has been sectioned serially (see column 5).

† For the length of the chloroplast its maximum extension within the serial sections was used. To determine the chloroplast volume, the formula for the rotating ellipsoid has been used, which implies that a chloroplast is circular in outline if viewed from above.

‡ The sectioned volume was determined by dividing the actually sectioned part of total chloroplast thickness (i.e. numbers of sections times thickness of single section of about 100 nm) by the maximum extension of the chloroplast within the serial sections considered. For suggested chloroplast shape, see footnote † above.

E, eudiploid; T, trisomic.

The shape of the DNA-structures is often influenced by starch grains (Figs. 2, 6, and also fig. 5 in Herrmann & Kowallik, 1970*b*). It seems noticeable that starch grains always appear closely associated with DNA-structures (Herrmann, 1970*b*, p. 87; Mikulska & Odintsova, 1970).

Spatial arrangement of DNA in plastids

Electron-microscopic serial sectioning of small plastids revealed that DNA-regions are usually stacked (Herrmann & Kowallik, 1970*b*). In autoradiographs this feature was regarded as the most probable reason for a relatively high number of silver grains over small plastids (Herrmann, 1970*b*; Herrmann & Kowallik, 1970*b*). As a control,

however, extensive series of sections through fully grown chloroplasts were still lacking at that time.

After evaluation of 29 different series of sections we conclude that stacking of DNA-structures in fully grown chloroplasts is less frequent, even if the matrix regions, in which the DNA is embedded, partly overlap (Figs. 2, 6, 7). Our former conclusion is thus further confirmed. Although in mature chloroplasts the DNA-regions are situated further apart from each other, it is nevertheless difficult to make an exact distinction between single DNA-regions of one chloroplast because of the more elaborate structure of the inner membrane system, of branching of DNA-regions, and of connexions between them. Therefore it is difficult to give an exact number of individual DNA-regions per chloroplast (see Table 1, and Discussion).

Number of DNA-regions per chloroplast

Undoubtedly the fully developed chloroplast exhibits more DNA-containing regions than a differentiating plastid (Figs. 1, 2, 5, 7; Table 1, and figs. and table in Herrmann & Kowallik, 1970*b*). This can already be concluded from partly sectioned chloroplasts. However, there are not enough series available to estimate the variation of numbers of DNA-regions in distinct size classes of chloroplasts. In spite of this the numbers of DNA-regions obtained by autoradiography (Herrmann, 1969, 1970*b*; Herrmann & Kowallik, 1970*b*) can be confirmed in broad outline; in some cases the number of regions per organelle seemed to be even greater than the number obtained by autoradiography (Fig. 5). No differences have been obtained between trisomic plants and eudiploid plants with respect to the numbers of DNA-regions for a comparable chloroplast size (Table 1, see also Herrmann, 1970*b*).

Size of DNA-containing structures

It is usually not possible to compare the volumes of DNA-regions in fully differentiated chloroplasts (compare Material and Methods); yet our series indicate a marked dissimilarity of DNA-quantity per region. The differences refer to both the spatial extension and the relative electron density of the DNA-containing structures.

We observed a few chloroplasts with compact DNA-structures similar to those of etioplasts (Herrmann & Kowallik, 1970*b*) which permitted a volumetric evaluation of the DNA-regions (Fig. 5). The results are comparable to those obtained for etioplasts. The measured volumes of such DNA-regions are: 0·013, 0·03, 0·03, 0·03, 0·05, 0·06, 0·06, 0·07, 0·09, 0·10, 0·10, 0·10, 0·11, 0·12, 0·18, 0·19 μm^3 (sectioned chloroplast volume about 14 μm^3). However, one cannot assume with certainty that the volumes of individual DNA-regions are proportional to their DNA-content.

An additional and independent method for estimating the relative DNA-content per region may be applied to those chloroplasts which have been sectioned tangentially and which exhibit the DNA in suitable fibrillar distribution, thus permitting determination of the length of the fibrils (Figs. 6, 9). It is obvious that the individual DNA-regions differ considerably in their DNA-content. With highly magnified photomicrographs, we evaluated an apparent total length of DNA-fibril(s) in the chloroplast of Fig. 6 of about 500 μm, i.e. about one-quarter to one-fifth of the average length of

DNA for this chloroplast size as deduced from biochemical analysis (Herrmann, 1972). This discrepancy may be explained by the fact that not all fibrils are of the diameter of a single DNA-molecule and that only about three-quarters of the total chloroplast has been sectioned (Table 1). If one establishes a relation between the individual DNA-regions, and if one assumes the relative value of 1 for the length of DNA of region *2*, then the total DNA-length of region *3* accounts for about 1·8 times, of region *5* for $>3{\cdot}5$ times, of region *1* for about 4·1 times, of region *6* for $>5{\cdot}5$ times, and of region *7* (not illustrated) for $>5{\cdot}6$ times that of region *2*.

In small plastids the volumes of the individual DNA-regions usually resemble each other (Herrmann & Kowallik, 1970*b*), although occasionally regions of aberrant sizes may be found. By way of example, in the chloroplast of Fig. 1 the DNA-regions measure 0·19 μm^3 (region *2*), 0·06 μm^3 (region *3*), 0·015 μm^3 (region *4*) and 0·009 μm^3 (region *1*).

DISCUSSION

For *Beta* chloroplasts, results from electron-microscopic and biochemical studies are now available which show the following:

(i) According to the serial sections of fully developed chloroplasts, there is no doubt that DNA in plastids of *Beta* – very small ones excepted (Herrmann & Kowallik, 1970*b*) – is arranged within several regions.

(ii) Among *Beta* chloroplasts there is a discrepancy between the DNA-amount per plastid (600–5500 μm, depending on the organelle sizes ranging from about 1·5 to 8 μm in diameter) and the amount of the monoploid information in the chloroplast genome (about 60 μm, as determined as kinetic complexity of the chloroplast DNA of *Beta vulgaris*; cf. part V of this series of papers, in preparation). The most reasonable explanation for this discrepancy would be that in one plastid there are several chloroplast genomes (more exactly 10–90) depending on the size of the organelle (Herrmann, 1970*b*, 1971, 1972).

If one combines both groups of data, some questions arise as to the genetic state of the DNA-regions: (i) Is the monoploid chloroplast genome scattered over several DNA-containing regions within the chloroplast matrix? Does it thus consist of several genophores?* (ii) Do only some DNA-regions contain the complete chloroplast genome, and thus do the remaining ones contain only a few nucleotide sequences which may be redundant? (iii) Does each DNA-region contain at least one complete chloroplast genome?

No direct methods are at present available to assign genetic units or DNA-molecules

* According to Piekarski (1937, cf. Rieger, Michaelis & Green, 1968), the nucleus-like structure in prokaryotic organisms is called a 'nucleoid' (originally applied only to bacteria). Instead of the term 'chromosome', Ris (1961, p. 112) coined the term 'genophore' to describe the linkage group in prokaryotes. If a DNA-containing structure contains the monoploid information in one DNA-strand, then the terms 'nucleoid' and 'genophore' would have the same meaning. It is, however, possible for a single nucleoid to contain several genophores, whether they are similar or not. In recent publications, the term genophore has been increasingly used. Unfortunately, it is not always meant in its original definition, but as a synonym of nucleoid (cf., for instance, Bisalputra & Burton, 1969, and the discussion of terminology in Bisalputra & Bisalputra, 1969).

to the DNA-regions of the chloroplast. There are, in addition, methodological difficulties which lie in the seriation of genetic units along DNA-molecules, and in the preparation and determination of unbroken DNA-molecules which in chloroplasts are certainly larger (Werz & Kellner, 1968; Woodcock & Fernández-Morán, 1968; Woodcock & Bogorad, 1970) than in animal mitochondria. The variable and high number of DNA-regions in fully differentiated chloroplasts is an additional difficulty.

Despite these difficulties, it seems justified to draw some conclusions from our data in comparison to other prokaryotically organized systems which seem more clearly characterized as to their genetic state, either by their response to distinct environmental conditions, or by biochemical or structural peculiarities. In dividing bacteria, usually 2 DNA-containing regions are visible. Each of these structures contains at least one complete genome, the information content of which being presumably considerably greater than that of the genetic unit of chloroplasts so far investigated (Wells & Birnstiel, 1969; Britten & Kohne, 1969; Tewari & Wildman, 1970; Bastia *et al.* 1971; Wells & Sager, 1971). Under certain conditions, bacteria may contain several genomes and DNA-regions also during interphase (Lark, 1966).

A structural arrangement of DNA very similar to that in chloroplasts has been observed electron microscopically in a *Pleurocapsa*-like blue-green alga (Beck, 1963), where a mother cell produces obviously as many endospores as there are DNA-containing regions. Beck therefore assigned a polyenergidic state to this cell type.

Evidence for variable amounts of DNA and different numbers of circular DNA-strands arranged within single DNA-regions (nucleoids) in mitochondria were reported by Nass (1969). There is considerable evidence that each DNA-region contains at least one genetic unit.

The bacterial as well as the blue-green algal and mitochondrial system mentioned seem to us to have much in common with our chloroplast system in so far as they contain more than one DNA-region. If one further realizes with regard to the bipartition of the chloroplast that an arrangement of entire genetic units within single DNA-regions would be certainly of selective advantage, each DNA-region would contain at least one complete genetic unit. This means that *each DNA-region can be considered as a nucleoid*; *consequently the chloroplasts of 'Beta vulgaris' would be of polyenergidic organization.** Our electron-microscopic data verify that it is difficult in a prokaryotic organization to prove a true separation of each DNA-region because of the absence of a structure similar to the nuclear envelope. Indeed, there are sometimes

* The terms 'polyenergidic organization' and 'polyploidy' are often confused. The term 'polyenergidic' was coined with reference to the term 'Energide' which had originally been introduced by Sachs (1892). It describes protoplasms containing more than one nucleus, as found in the orders Siphonales (*Derbesia*), Heterosiphonales (*Vaucheria*), Siphonocladales (*Cladophora*), or certain fungi (slime moulds, phycomycetes). In a more general meaning, it would implicate the localization of multiple amounts of DNA, each quantity within a different part of the cell. In this sense, the term 'polyenergidic' has been applied more recently to prokaryotic organisms (blue green algae: Fuhs, 1958; Beck, 1963; Geitler, 1963), and is now being used for chloroplasts (Herrmann, 1969) because of their apparently equivalent organizational state. On the other hand, in a polyploid organelle multiple amounts of DNA would be localized within *one* place, as known, for instance, from polyploid nuclei. Applied to chloroplasts, this would mean that each nucleoid (cf. footnote, p. 362) contains several genophores.

– especially in tangentially sectioned DNA-regions – small fibrils virtually connecting 2 neighbouring regions (Fig. 6). In these cases the term 'polyenergidic organization' *sensu stricto* would not be applicable. However, there are numerous DNA-regions which appear to be completely separated, as shown by spatial analysis of the thylakoid system (compare, for instance, regions *3* and *4* in the chloroplast of Fig. 1).

The type of a really polyenergidically organized chloroplast was recently demonstrated in the dinoflagellate *Prorocentrum micans* (Kowallik & Haberkorn, 1971), where many (up to approximately 100) DNA-containing regions appear clearly separated from neighbouring regions by lamellate thylakoid stacks, as has been worked out by 3-dimensional reconstructions following serial sectioning.

According to our data, the ratio between the number of chloroplast genomes per organelle (Herrmann, 1971, 1972 and the number of DNA-containing regions per organelle is about 4–8. Provided that genetic units in DNA-regions are arranged more regularly, as discussed above, plastids of *Beta* would be genetically polyvalent in a second way: the individual nucleoids can be *polyploid, and moreover, polyploid to varying degrees*. This type of chloroplast would thus be comparable to a heterokaryotic state on the level of the eukaryotic cell.

DNA-amounts per plastid as well as the degree of polyenergidic organization and the size of organelle are correlated in *Beta* (Herrmann, 1970*a*, *b*). Since the size of the organelle depends on the developmental state of the leaf, one can conclude that the degree of polyenergidy (and thus the degree of genetic polyvalency) is dependent upon the development of the plastids. In contrast, mitochondria of *Beta* in general contain only one nucleoid, independent of the age of the leaf (author's unpublished results).

The question is still open to discussion as to whether the results regarding genetic polyvalency of *Beta* chloroplasts can be applied to chloroplasts of other plants as well. Extensive series of sections of chloroplasts of other species have not yet been published (cf., however, Diers & Schötz, 1966; Sprey, 1968). Electron micrographs of single sections through plastids of algae and higher plants, however, show fibril-containing electron-transparent areas in similar arrangement to those of *Beta*. Therefore, a polyenergidic organization of plastids seems to be quite common among plants of different relationship.

Division of plastids in spite of inhibited DNA-synthesis further indicates genetic polyvalency (Boasson & Laetsch, 1969). However, it remains to be shown in this case whether all of the division products of the plastids still contain DNA. This possibility must be taken into consideration, since Woodcock & Bogorad (1970) reported the absence of DNA in about 70% of the plastids of *Acetabularia*. Although marked differences in the DNA-content among the rest of the plastids of *Acetabularia* were to be found, the peculiar plastid system of this alga would not permit an unlimited comparison with that of *Beta* and other plants.

Early investigations of ultraviolet-irradiated plastids of *Euglena* indicated a polycentric arrangement of DNA (Lyman, Epstein & Schiff, 1961; Schiff, Lyman & Epstein, 1961) which seems to resemble morphologically the polyenergidic state in *Beta* chloroplasts. On the other hand, evidence for polyploid chloroplasts was provided

by Surzycki, Goodenough, Levine & Armstrong (1970) following incorporation of bromodeoxyuridine into the chloroplast-DNA of *Chlamydomonas reinhardi*. As a result only very few chloroplast-DNA mutations were obtained, and it was therefore suggested that single gene mutations would be masked, thus indicating a highly polyploid or redundant genome. A structurally peculiar type of chloroplast nucleoid is known from *Sphacelaria* (Bisalputra & Bisalputra, 1969) and *Ochromonas* (Slankis & Gibbs, 1968). According to the terminology used in this report, the ring-shaped nucleoids would be polyploid in different degrees, if the volume of each nucleoid, depending on the size of the organelle considered, would approximately reflect its DNA-content.

The relation between the number of DNA-regions (i.e. the degree of polyenergidic organization) and the development-dependent chloroplast size in numerous plants (*Zea*: Jacobson, 1968; *Hordeum*: Gunning, 1965) seems to be comparable to that in *Beta*. This similarity could also account for the relation between DNA-content and size of these organelles, as has now been demonstrated for the chloroplasts of *Beta vulgaris*.

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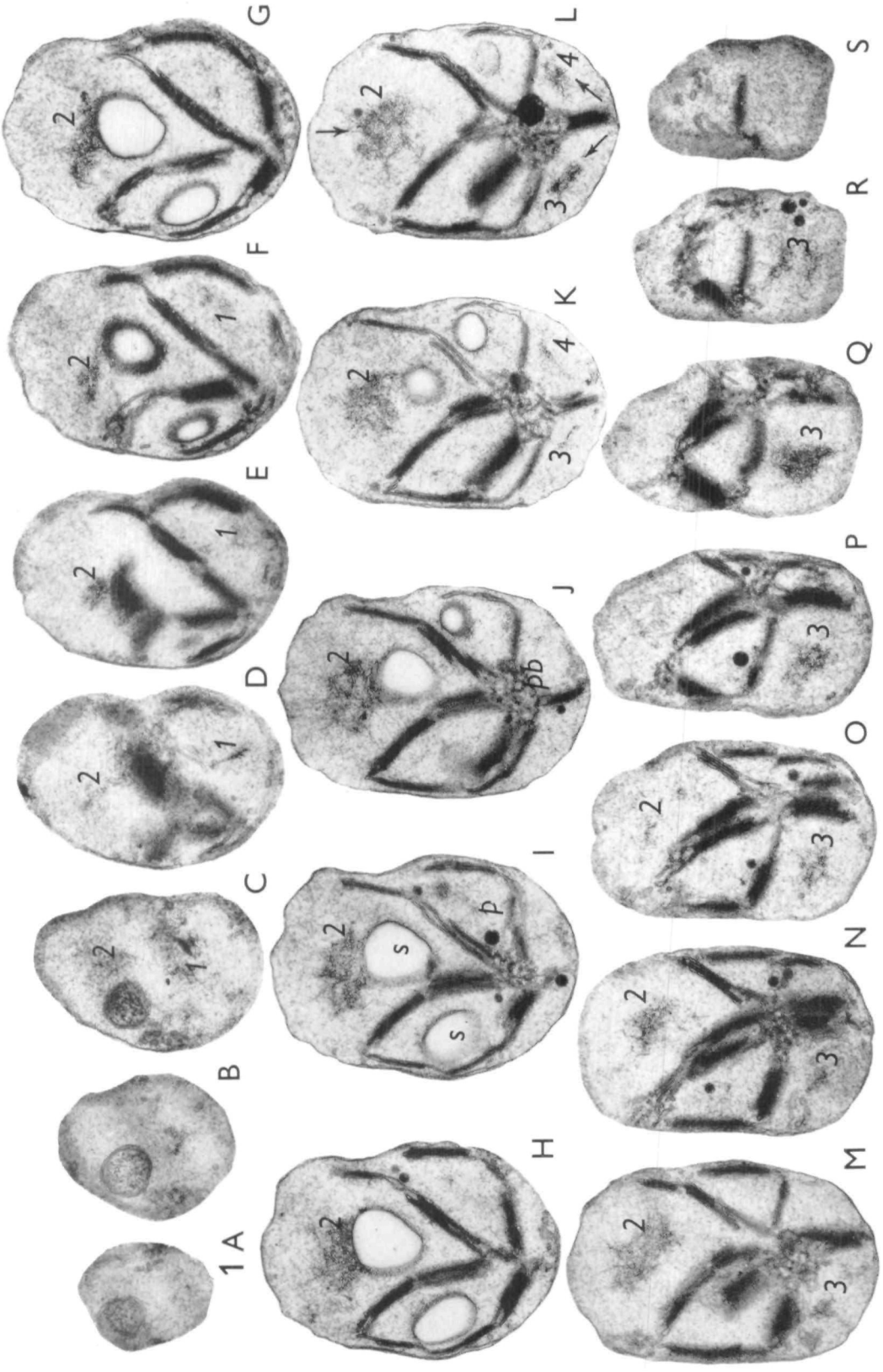
Fig. 1. Etioplast from a differentiating leaf after incomplete trypsin treatment. Four visible DNA-regions which appear clearly separated by thylakoid lamellae, can be followed through the serial sections. Note the chloroplast envelope which is not, as in fully differentiated chloroplasts, visibly attacked by the enzyme. The heavy contrast of the DNA-structures (arrows) is presumably caused by adsorbed proteinaceous digestion products. *p*, plastoglobule; *pb*, prolamellar body; *s*, starch. × 18000.

Fig. 2. Part of serially sectioned chloroplast, showing several matrix regions of different extension containing fluffy or fibrillar DNA (cf. also Fig. 7). Starch grains appear in close association with DNA-structures. *ce*, fragmented chloroplast envelope; *s*, starch. × 13000. *Note:* detail of Fig. 2v placed ahead of the series to show 'smooth' DNA-fibrils (arrow) and fluffy DNA-structures within incompletely digested regions *1*, *2* and *5* more clearly. × 40000.


1A
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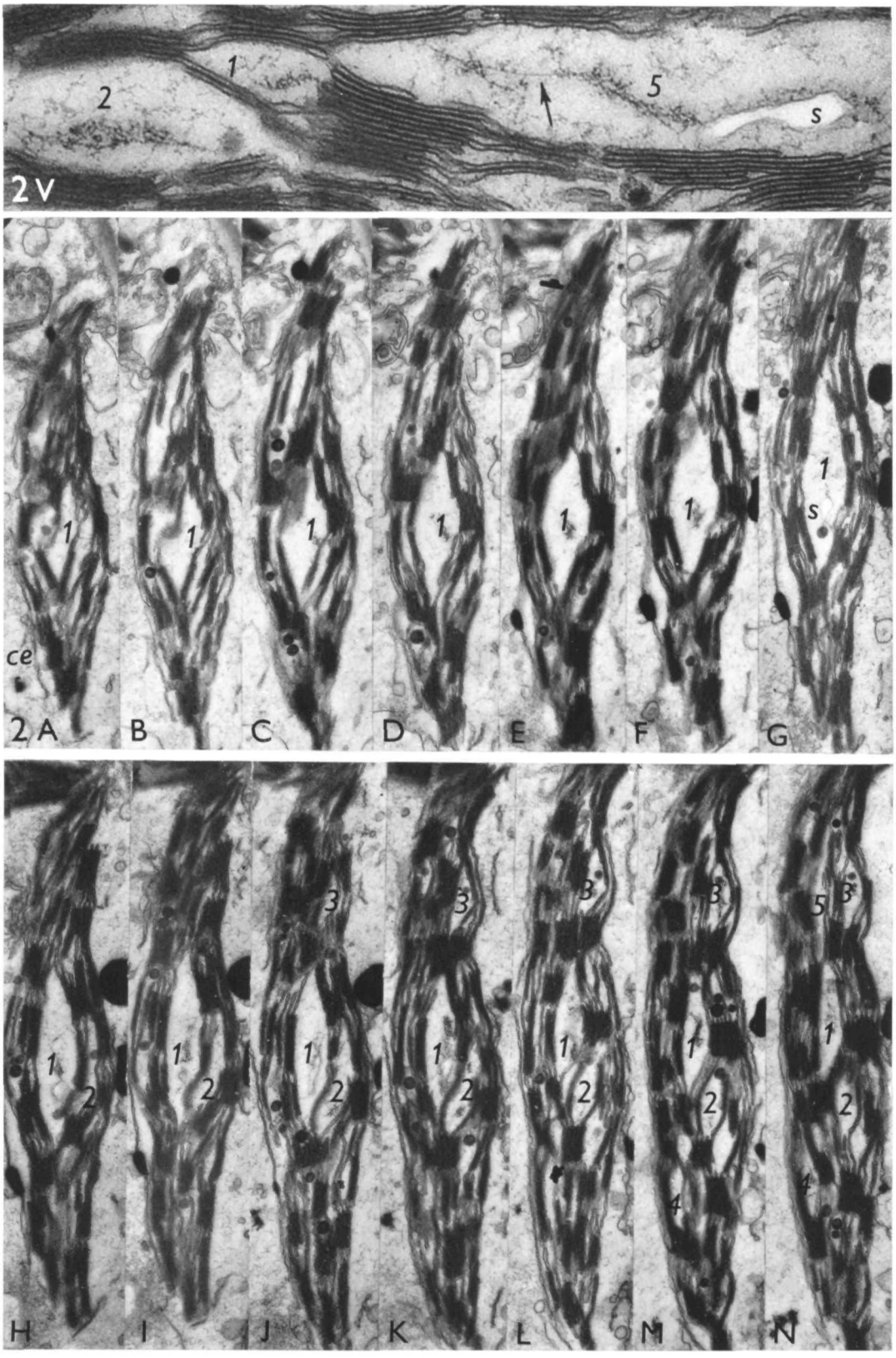


Fig. 2. For legend see p. 368.

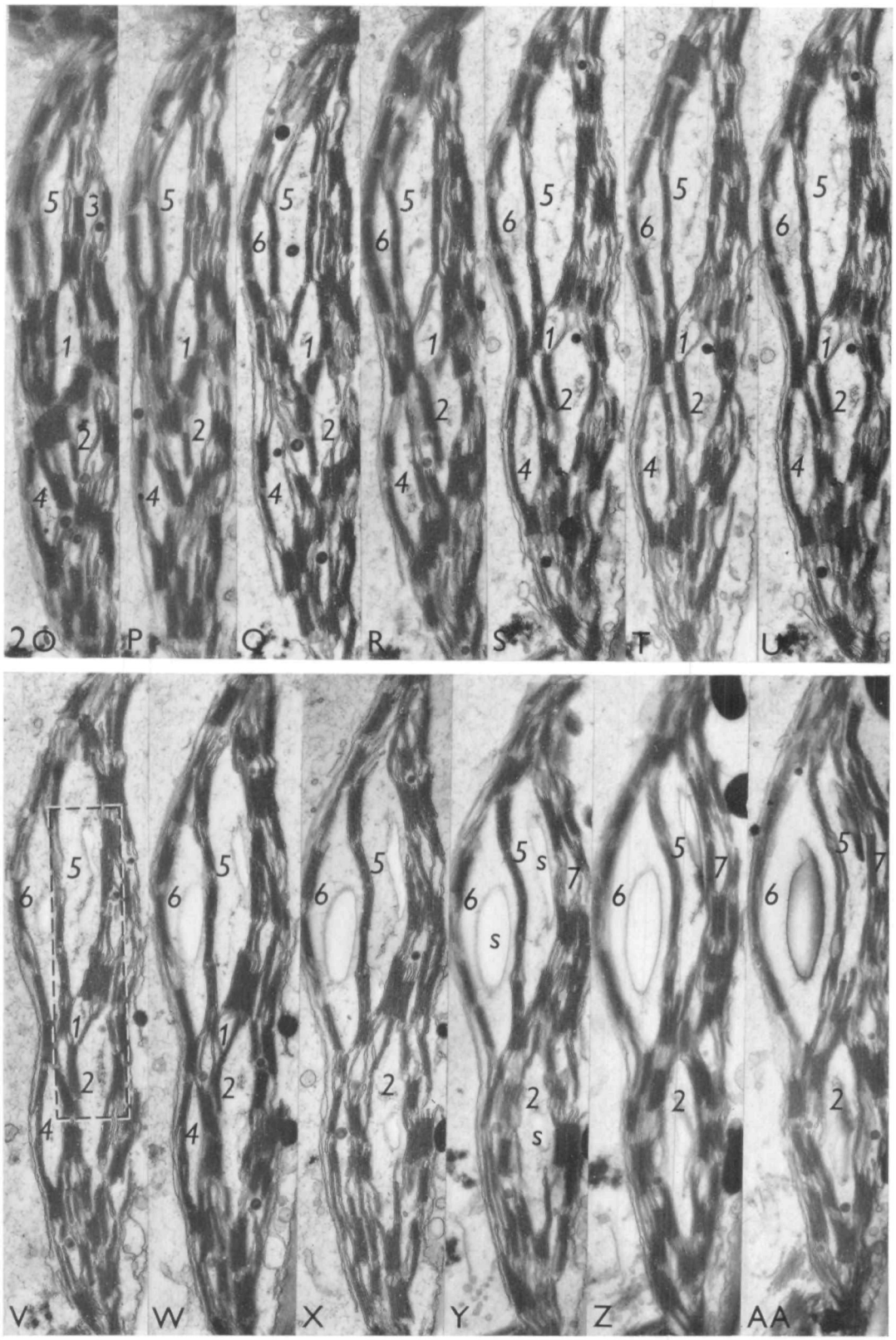


Fig. 2. For legend see p. 368.

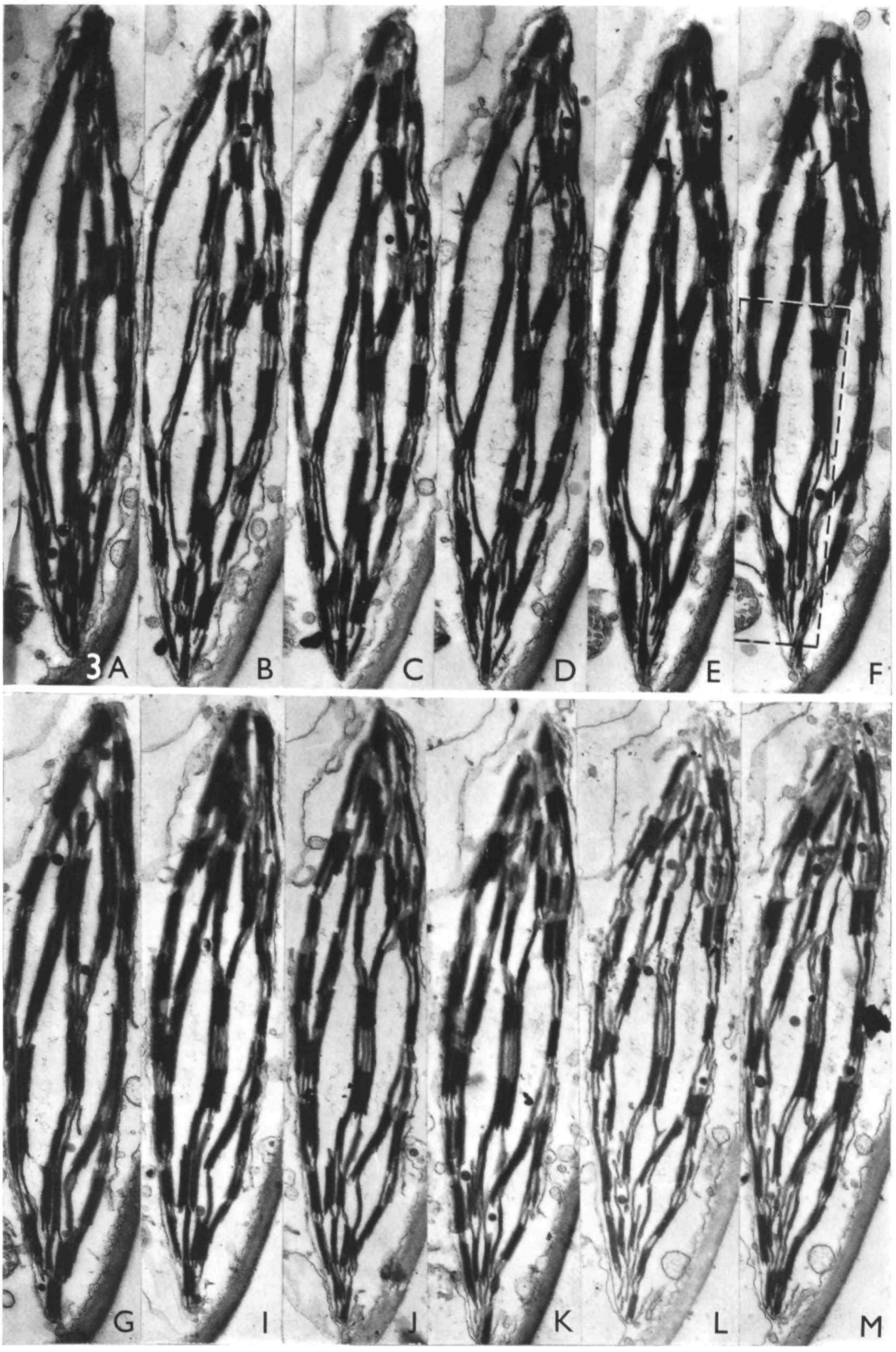
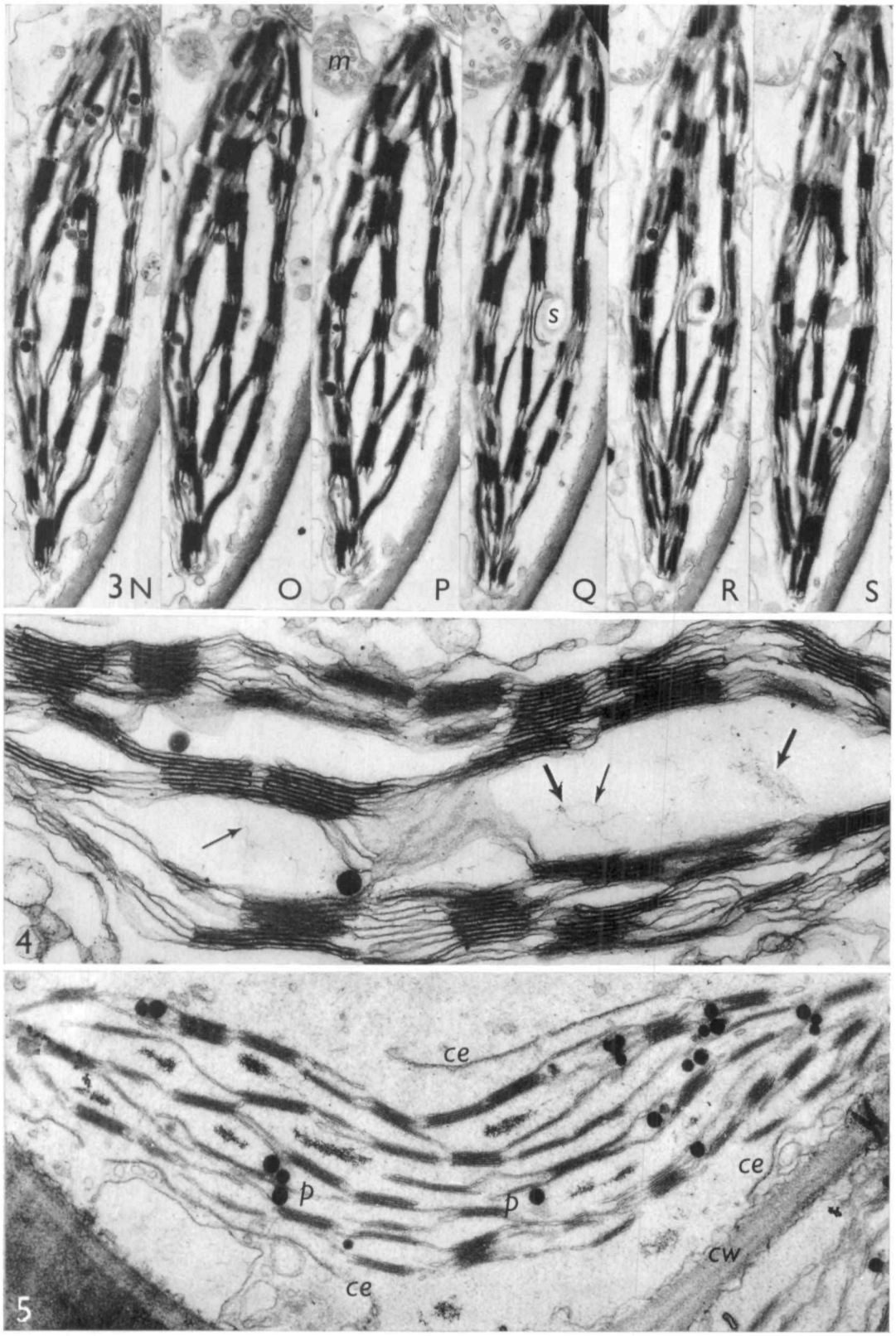


Fig. 3. For legend see p. 374.



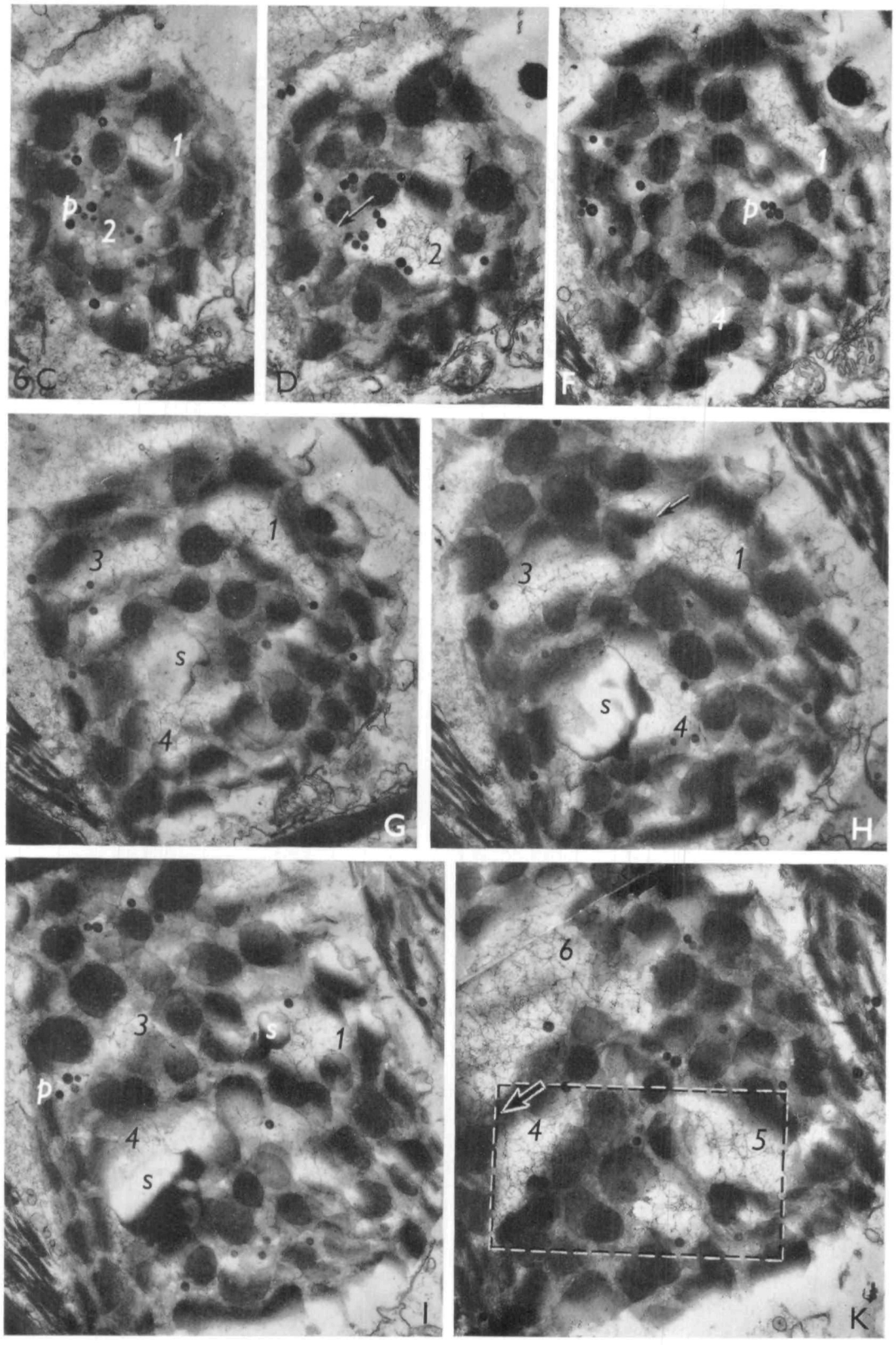
Figs. 3–5. For legend see p. 374.

Fig. 3. Serial sections of a chloroplast (section H omitted), showing complete digestion of both cytoplasm and chloroplast matrix. Thus only distinct DNA-fibrils remain within the electron-transparent matrix regions (cf. also Fig. 8). *m*, mitochondrion; *s*, starch. × 14000.

Fig. 4. Fully digested chloroplast more highly magnified. The thylakoids are visible in distinct outline even if sectioned tangentially. Thin DNA-fibrils, about 3 nm in diameter, are irregularly arranged within electron-translucent areas (thin arrows) and show many (probably artificial) connexions to thylakoid membranes. Fluffy material presumably of proteinaceous origin appears to be attached to the DNA-fibrils (thick arrows). × 32000.

Fig. 5. Example of a serially sectioned chloroplast after incomplete tryptic digestion (granular background). Within several matrix regions there are 9 well contrasted DNA-structures of fluffy appearance. Chloroplast envelope (*ce*) already fragmented. *cw*, cell wall; *p*, plastoglobule. × 20000.

Fig. 6. Serial sections of a fully differentiated chloroplast. The section plane is parallel to the granal thylakoids which are thus seen with circular outline (sections A, B, E, J, L not illustrated). As a result of tryptic digestion, the chloroplast envelope has disappeared. DNA appears as a network of fine fibrils which preferably occur within centres (centres marked *1–6*). Single fibrils occasionally traverse into adjacent stroma regions (thin arrows) without forming distinct centres, but may also possibly connect neighbouring centres such as *4* and *6* in the horizontal direction (thick arrow), and *3* and *6* in the vertical direction (cf. also Fig. 9). *p*, plastoglobule; *s*, starch. × 12000.


1
P
2
6C
2
D
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4
F
1
3
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H
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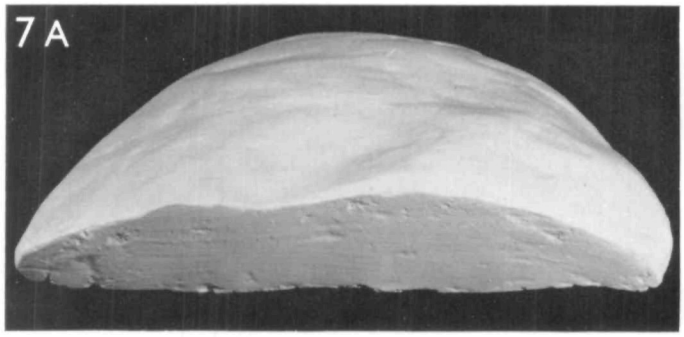
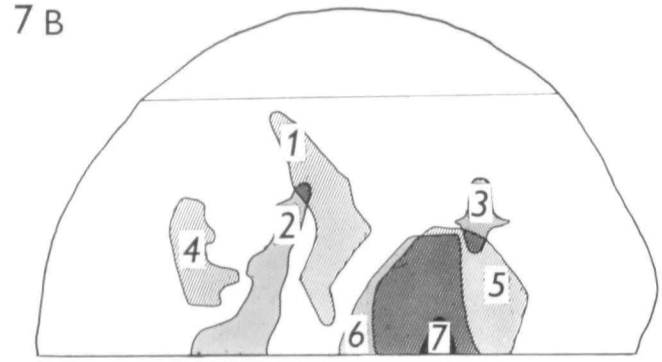
1
P
4
S
I
6
4
5
K

Fig. 7. Reconstructions of the serially sectioned chloroplast of Fig. 2: outer morphology of the sectioned part of the chloroplast (7A), spatial arrangement of DNA-containing matrix regions (7C) and their 2-dimensional projections (7B).

Fig. 8. Detail of Fig. 3F, more highly magnified to show the fibrillar DNA more clearly within completely digested stroma regions (arrows). Note also the absence of any cytoplasmic content except membranes and vesicles as a result of tryptic digestion. A few DNA-fibrils also visible within a mitochondrion (arrow). × 35000.

Fig. 9. Detail of Fig. 6K, showing the DNA regions *4* and *5* at higher magnification. Note the increased transparency of the tangentially sectioned thylakoid stacks. The DNA-fibrils partly appear like strings of pearls, possibly the result of adsorption of proteinaceous digestion products. *p*, plastoglobule. × 35000.


7 A


7 B
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2
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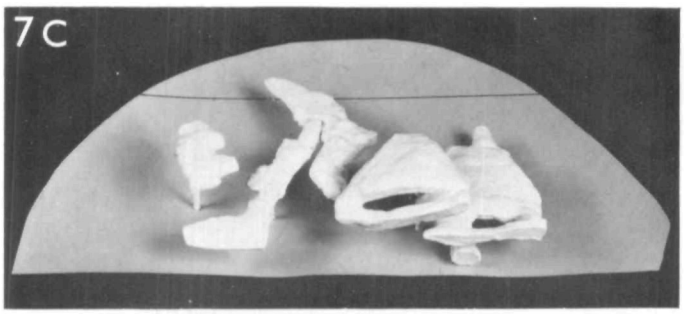
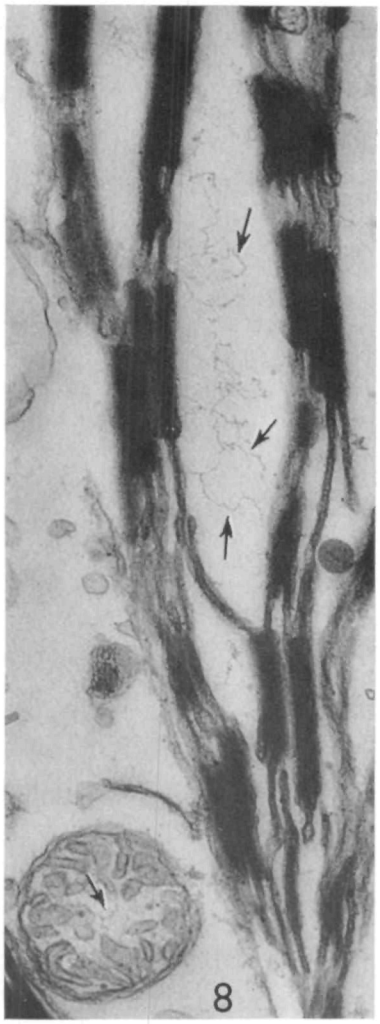
7


7 C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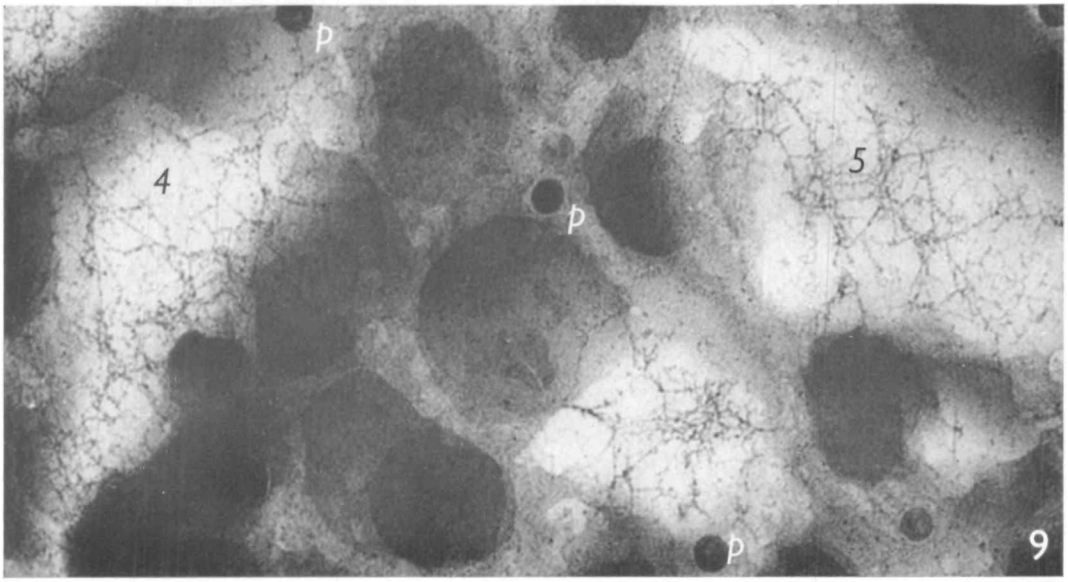
P
4
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5
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