

# Formation of Proplastids in the Apical Meristem of Wheat

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## Abstract

The apical meristem of the growing point of plants contains proplastids, precursors of chloroplasts. The main attention of investigators was paid to the transformation of proplastids into chloroplasts. The formation of proplastids of the apical meristem of wheat seedlings was investigated in the present work and described for the first time in the scientific literature. Ultrastructural images of apical meristem areas showed that the formation of the proplastide body includes several stages: localization of plastid DNA in the cytoplasmic matrix in close contact with cytoplasmic ribosomes, the formation of membrane vesicles containing plastid DNA, the step-by-step filling of these vesicles with dense contents, and the formation of mature proplastids.

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## Introduction

The apical meristem of the growing point of plants contains proplastids, precursors of chloroplasts. Proplastids are colorless spherical organelles 1–2  $\mu\text{m}$  in diameter surrounded by a membrane. These organelles are composed of a dense matrix, ribosomes, and plastid DNA. They have no inner membranes, i.e. thylakoids. The studies on the ultrastructure of the apical meristem of a plant shoot are few. In the review of Pyke [1] on the biogenesis of plastids, only a few papers are devoted to proplastids, and even these studies have been performed mainly on the apical meristem of roots.

The results of studies on the apical meristem and leaf primordia of a variety of plants have shown that proplastids seen on micrographs already contain thylakoids, although they are few in number [2-5]. The main concern of investigators was with the transformation of proplastids into chloroplasts, and the object of investigations was often young leaves of three- to seven-day-old cereal shoots at the base of which the intercalary meristem is located. In this meristem, proplastids already have a flattened form, containing single thylakoids and prolamellar bodies, and are actually young chloroplasts [6-8].

The apical meristem of the growing point of wheat seedlings is hidden at the base of a plant, is buried into soil, and is tightly covered with the bases of two to three leaves. Therefore, this meristem can contain proplastids that still have no internal membranes.

The ultrastructure of the apical meristem of wheat seedlings has not been studied earlier. In this work, the images of the formation of proplastids of the apical meristem of wheat seedlings were obtained and described for the first time in the scientific literature.

## Materials and Methods

The object of the investigation was the 12- to 14-day-old seedlings of the wheat *Triticum aestivum* L. Seedlings were grown in vials with soil under natural illumination at room temperature (20–23°C) to an age of 10–12 days. Samples from five independent experiments were examined. All samples showed a similar ultrastructural organization.

The materials were fixed and prepared for examination by standard methods. Pieces of shoots adjacent to the caryopsis 2 to 3 mm in height were fixed in a 3% glutaraldehyde solution on phosphate buffer (pH 7.4) for 2 h followed by the postfixation in a 1%  $\text{OsO}_4$  (Reakhim, Russia) solution. After dehydration in alcohols of increasing concentrations 50-100% and 100% acetone, samples were embedded in Epon-812 (Fluka, Germany).

Ultrathin sections were contrasted with a saturated aqueous uranyl acetate (Sewa, Czech Republic) solution and 0.25% lead citrate (British Drug Houses, England) by the conventional methods and examined in a JEM-100B electron microscope (Jeol, Japan) under an accelerating voltage of 80 kV.

## Results

Figures 1 and 2 show the sections of cells of the apical meristem of the growing point at different magnifications. In the cytoplasm of meristematic cells, along with typical proplastids, numerous single-membrane vesicles containing fibrils of plastid DNA (arrows 1 in the photomicrograph) are seen. At some places, thin single fibrils form closely contacting conglomerates. In the center of the conglomerate, two ring-shaped structures (arrows 2 in the photomicrograph) are seen (Fig. 2a). The vesicles are 1–2  $\mu\text{m}$  in diameter and are filled to a different degree with a dense content (Figs. 1–3). These images can be considered to reflect the process of the step-by-step formation of proplastids from membrane vesicles.

During the formation of a proplastid body, the single-membrane envelope remains perforated until the complete maturation of the proplastid (Figs. 3a–3e). At the first stage of the formation of a proplastid, the membrane of the vesicle, with plastid DNA inside it (arrows 3 in the photomicrograph), is almost closed (Figs. 3a and b). In the early stages internal cavity of the vesicle and the cytoplasm are in direct contact in a small region where the membrane is disrupted (Fig. 3a). At later stages, the area of contact between the inner cavity of the proplastid body and the cytoplasm increases (Figs. 3c–3e). When the proplastid body is almost completely developed, the membrane of its

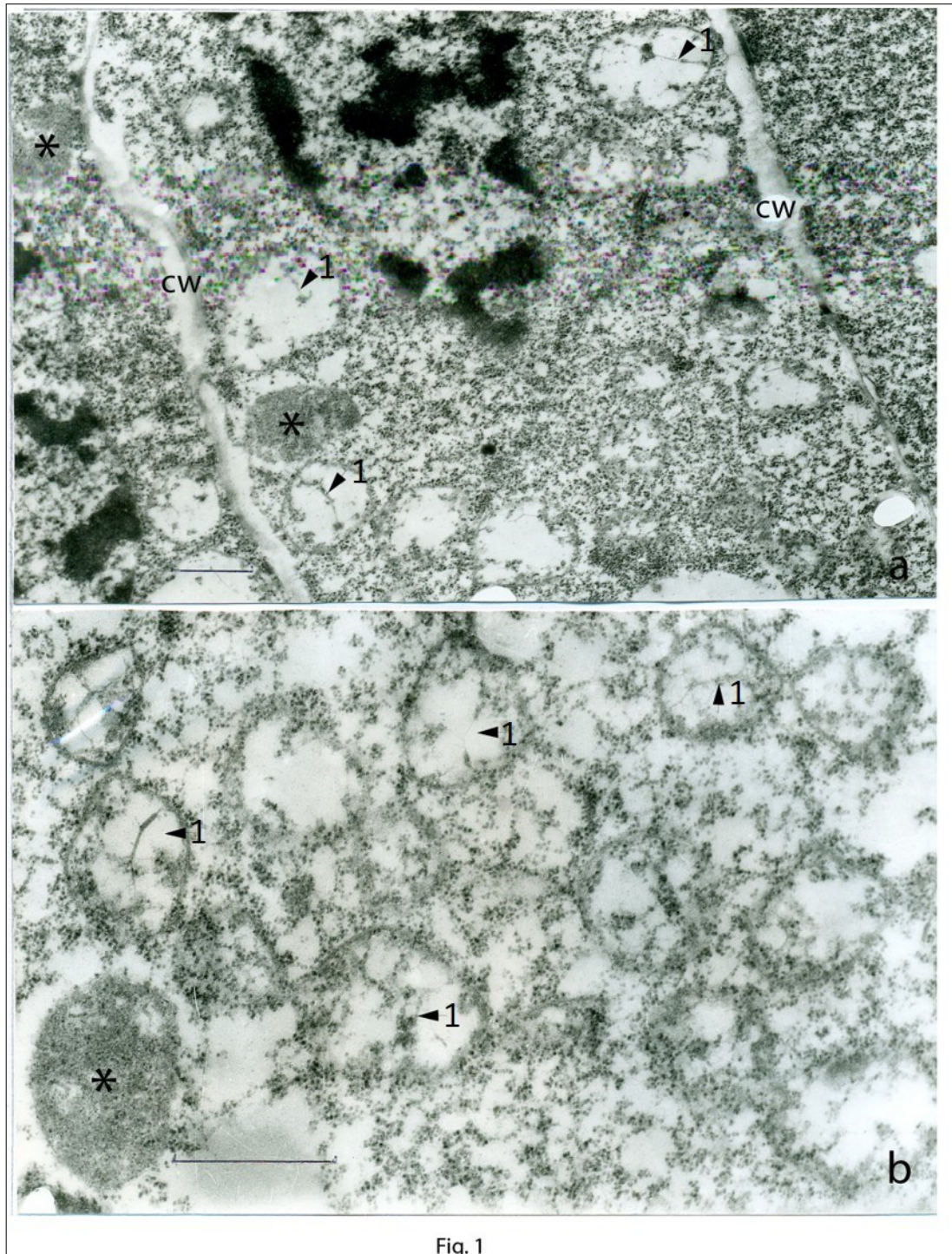


Fig. 1. Cells of the apical meristem of the growing point. A general view (a) and a region of the cell at a large magnification (b). Membrane vesicles contain plastid DNA filaments (arrows 1). The developed proplastid is shown by an asterisk; cw, cell wall. Scale bar = 1  $\mu$ m.

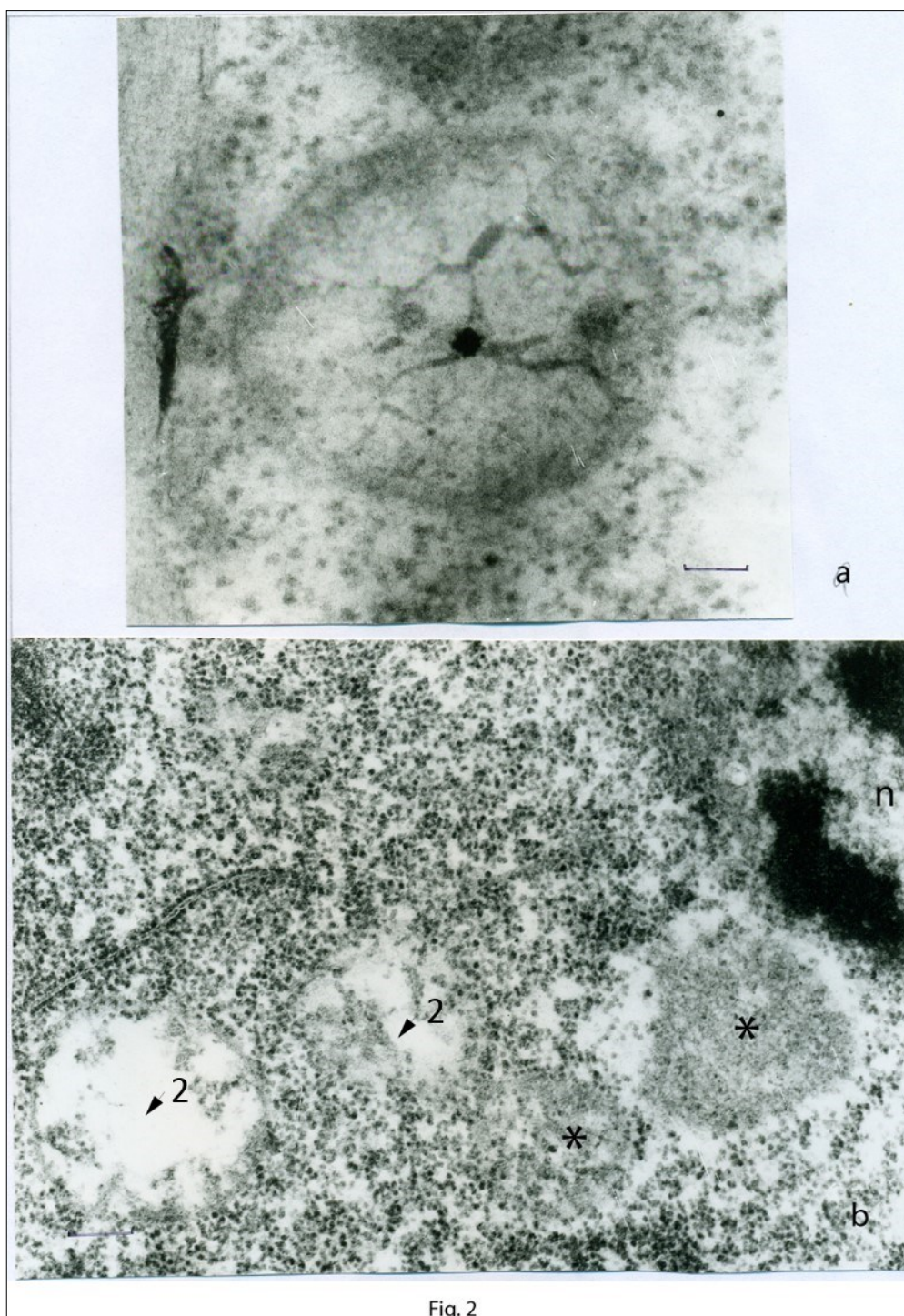


Fig. 2

Fig. 2. Regions of the cells of the apical meristem at a large magnification. Proplastid contained plastid DNA (a) and proplastids at different degree of maturation (b). The developed proplastids are shown by asterisks; plastid DNA is shown by arrows 2. Scale bar = 0.2  $\mu$ m.

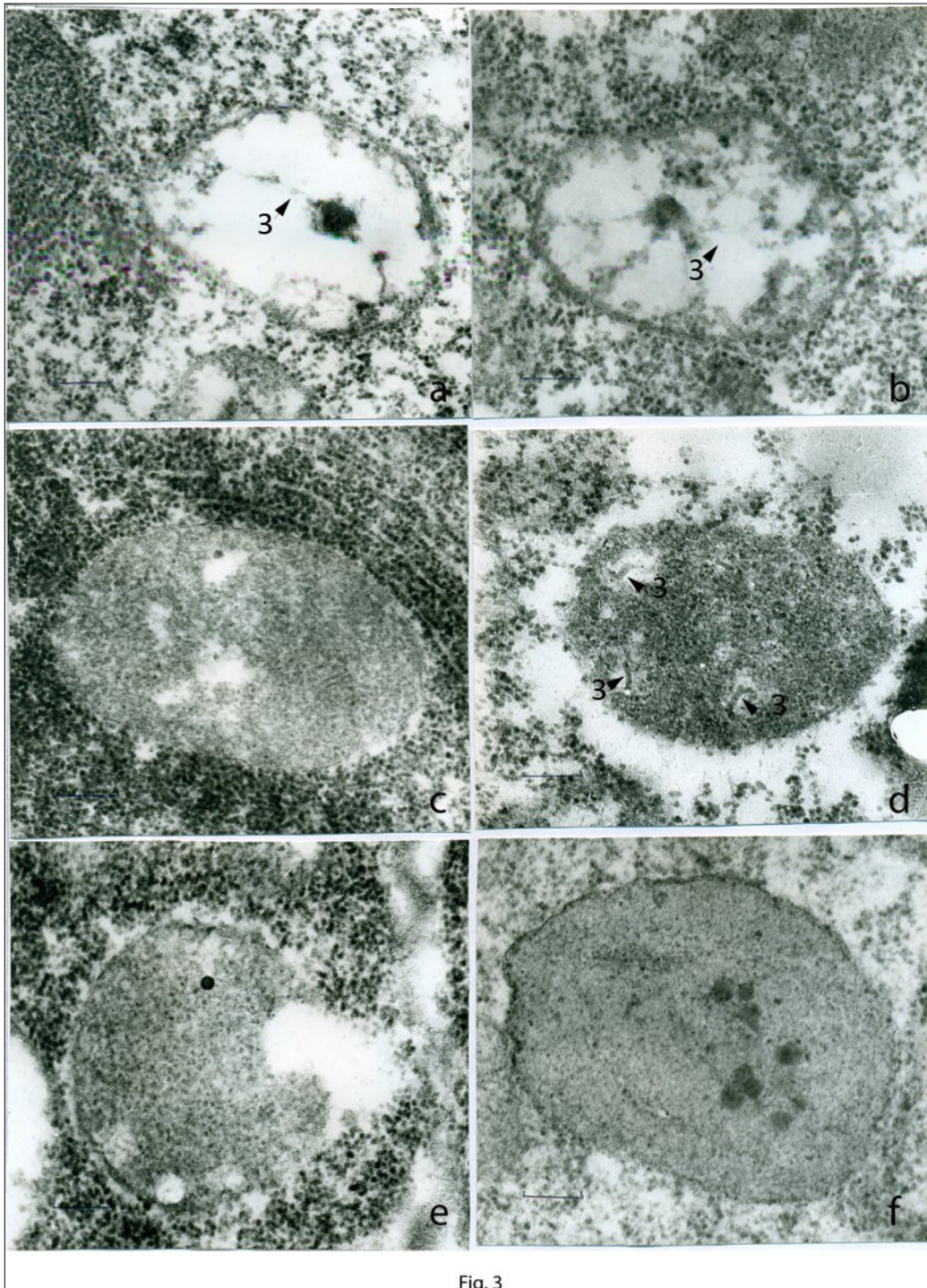


Fig. 3

Fig. 3. Stages of formation of proplastids in the apical meristem (a-f): a, b – the onset of the formation of the proplastid body. Membrane vesicles contained plastid DNA filaments are shown by arrows 3. c-e – developed proplastid bodies. Fragments of plastid DNA (arrows 3) and cavities in the matrix of proplastids are seen. f – a mature, completely developed proplastid surrounded by a dense membrane and containing a dense matrix, plastid ribosomes, and plastoglobuli. Scale bar = 0.2  $\mu$ m.

envelope is perforated to a greater degree, and a direct contact between the proplastid body and the ribosomes of cytoplasm is seen (Fig. 3). The density of ribosomes in the cytoplasm of meristematic cells is very high, up to about  $3000/\mu\text{m}^2$ . A completely developed proplastid surrounded by a dense closed membrane contains a dense matrix, plastid ribosomes, and plastoglobuli (Fig. 3f).

The plastid DNA at a large magnification (arrows 4 in the photomicrograph) is shown in Figure 4. The proplastid, with plastid DNA inside, is surrounded by a perforated membrane. The matrix of the proplastid is reduced, and cytoplasmic ribosomes go into the proplastid body (Fig. 4a). The plastid DNA seen among cytoplasmic ribosomes (Fig. 4b). The contours of the proplastid itself are poorly distinguished, and the reduced membrane of the proplastid envelope is retained only partially (Fig. 4b). Figure 4c shows plastid DNA filaments localized in the matrix of the cytoplasm; to the right, a fragment of the membrane nucleus (n) is seen; the nucleus envelope cannot be distinguished.

The micrographs can also be considered as reflecting the process of proplastid degradation as a result of which plastid DNA becomes submerged immediately into the cytoplasm of the meristematic cell.

## Discussion

Plastid DNA visible at the ultrastructural level has a clearly defined morphology. It is represented by a complex of fibrils 2.5–3.0 nm in diameter, which in certain places fuse into dense conglomerates [9]. The region occupied by plastid DNA is a nucleoid, which is identified by the level of fluorescence [10]

Membrane vesicles similar to those obtained in our study can be seen in micrographs shown in other works. These type of vesicles are found on the sections of ovicells or meristems of various plants; however, either their status is not discussed [5, 11, 12] or they are considered to be vacuoles or degraded proplastids [13].

Japanese researchers suggested to divide proplastids into two groups: plastid initials and mature plastids [14, 15]. However, micrographs presented in their papers show that "proplastid initials" containing plastid DNA and a dense matrix in meristematic cells of

plant buds, potato stolons, and wheat seedlings are bodies surrounded by two membranes. These bodies can be called true developed proplastids rather than initials. "Mature" proplastids presented in these works contain internal membranes and are actually young chloroplasts. The differences in the enzymatic activity between "initial" and "mature" proplastids revealed by Akita and Sagisaka [14] are in fact the differences between true proplastids and young chloroplasts.

The transition from a proplastid to a young chloroplast is very quick, and this process in meristematic cells is triggered even by weak illumination. Meristematic cells in shoots of various plants contain as a rule plastids with thylakoids [2, 15, 16]; in this case, the degree of the development of thylakoids depends on the localization of cells in the apical meristem [17].

Electron transparent single-membrane vesicles containing plastid DNA, observed in the present work, can be called plastid initials. They are more similar to "particle initials" in the filicine egg cells, which were given this name by Bell et al. [18], than to those described by Sagisaka and Kuroda [15].

Bell et al. [18] presented micrographs from which it can be assumed that plastid initials are budded off immediately from the nucleus envelope. Based on the images presented by Sagisaka and Kuroda [15], it can be proposed that plastid initials are formed by protrusion and separation from mature proplastids.

In the present study, the division of neither developed nor developing proplastids was observed in the cells of the apical meristem. The localization of plastid DNA immediately in the matrix of the cytoplasm in close contact with cytoplasmic ribosomes shown in micrographs suggests that in this state the replication of plastid DNA in the cytoplasmic matrix is possible. A great number of plastid DNA copies are covered by a membrane and generate those plastid initials that subsequently develop into mature proplastids.

Boffey and Leech [19] showed that the number of plastid DNA copies at the early stages of the development of wheat leaves is 800–1000 per plastid, and at later stages this number decreases to 300. This observation can be explained assuming that, at early

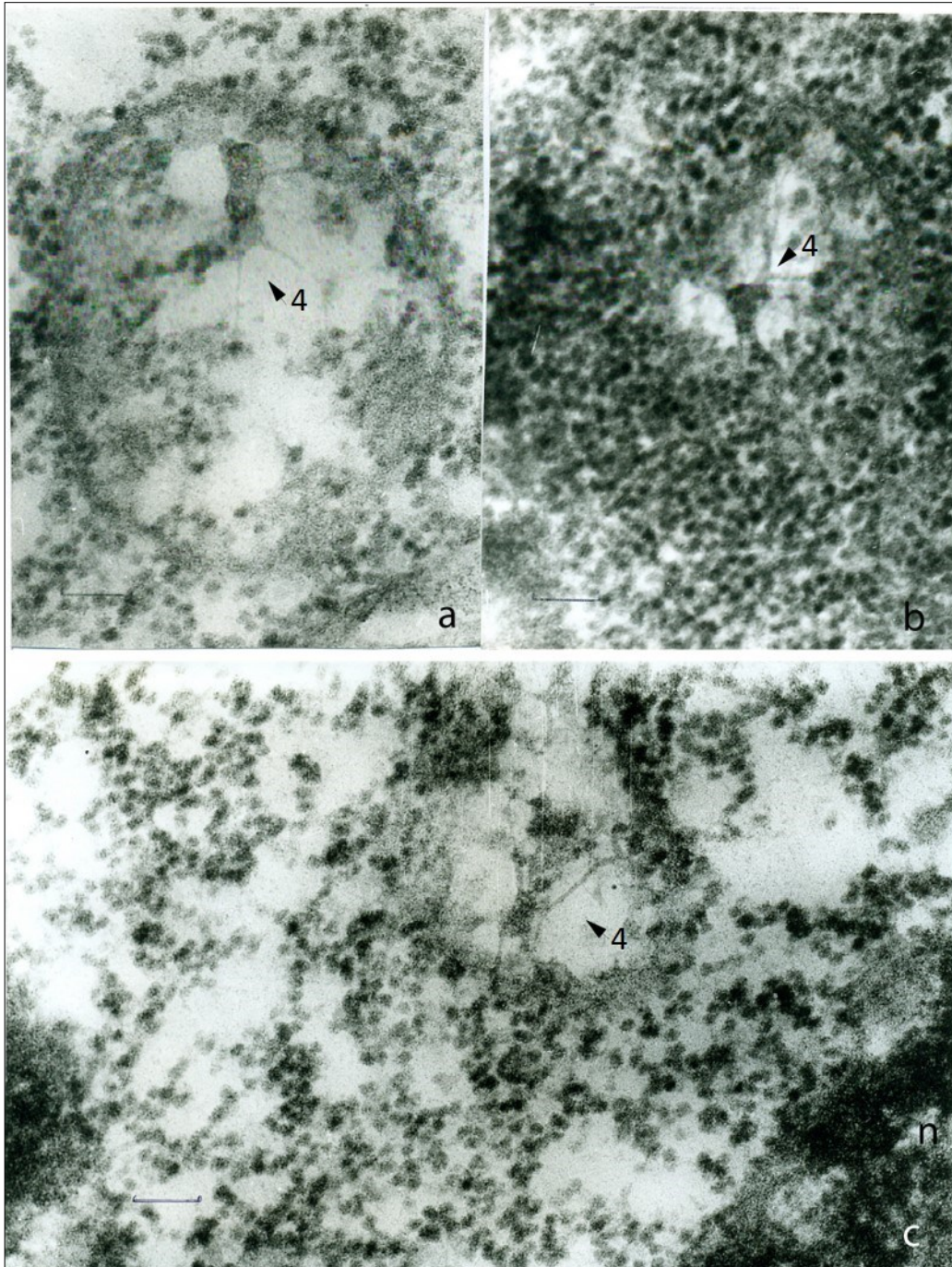


Fig. 4

Fig. 4. A region of the cytoplasm of apical meristematic cells (a–c). a – plastid DNA in an almost completely closed proplastid membrane. The matrix of the proplastid is reduced, and ribosomes of the cytoplasm are seen inside it. b – plastid DNA localized among the cytoplasmic ribosomes; a part of the membrane of the proplastid is seen (arrows 4). c – plastid DNA localized outside the proplastid, among cytoplasmic ribosomes; to the right is seen chromatin of the nucleus (n). Plastid DNA is indicated by arrows 4. Scale bar = 0.2  $\mu$ m.

stages of plant development, plastid DNA can exist outside plastids.

### Author Contribution Statement

GS designed and performed the experiment, analyzed the data and wrote the manuscript.

### Compliance with Ethical Standards

Conflict of Interest

The author declares no conflict of interest.

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