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## INTRANUCLEAR AND CYTOPLASMIC INCLUSIONS IN EPIDERMAL AND PARENCHYMA CELLS OF SIBERIAN PEA

*The nuclei and cytoplasm of growing epidermal and parenchyma cells of Siberian pea are characterized by the presence of spherical bodies. These structures are Feulgen-negative but stain positively with Methyl green — Pyronin and Azure B for the presence of RNA and with Fast green and Bromo phenol blue for basic proteins. At the ultrastructural level, the bodies consist of closely packed fibrils and are distinguished as morphologically changing structures. These inclusions are non-viral in nature and present in normal, healthy cells.*

### Introduction

A number of reports have appeared in recent years on the presence of various inclusions in plant cells [1—7]. Although many studies were undertaken on these formations, their structure and nature still remain the subject of investigations and discussions because of the variety of sources causing formation of these structures and similarity of inclusions with different origin. Intranuclear and cytoplasmic inclusions, present in a variety of plant species, may be observed in normal cells [4—7] as well as in virus infected cells [1—3]. The present work was therefore undertaken in order to investigate the distribution, number and size as well as the possible nature of the spherical inclusions that appear naturally during spring in the nuclei and cytoplasm of epidermal and parenchyma cells of Siberian pea plants.

### Material and methods

Epidermal and parenchyma cells of sepals, pedicels and petioles of normal, non-symptom Siberian pea plants (*Caragana arborescens* Lam.), growing in different Kiev parks, were used for the present study. The material was collected from the stage of the buds' unfurling till the petals' falling. To verify the presence of nucleic acids under light microscopy, the specimens were stained with 2% Methyl green — Pyronin aqueous solution, 0.2% Azure B solution in 0.1 M McIlvein buffer, pH 4.0 and the reagent of Feulgen without the preliminary fixation of the material. The presence of proteins was examined by staining with 0.1% aqueous solutions of Fast green, pH 8.0 and Bromo phenol blue, pH 4.5, also without the preliminary fixation. For the electron microscope study, the material was fixed for 2 h, at 4 °C, in 2.5% glutaraldehyde buffered, pH 7.2, with 0.1 M sodium cacodylate.

After thoroughly rinsing in the same buffer, the specimens were then postfixed overnight with 1% osmium tetroxide. Following dehydration in alcohol, the specimens were embedded in Epon 812. Ultrathin sections, stained with both uranyl acetate and lead citrate, were mounted on Formvar-covered single-hole grids and examined in a JEM 1200 EX electron microscope. To determine the possible nature of these inclusions, indicator plants (*Glycine max* var. Slava; *Phaseolus vulgaris* var. Contender and Derby; *Pisum sativum* var. Uladivsky; Russian beans; *Nicotiana tabacum* var. Samsun; *Chenopodium album*; *Vigna* and *Petunia hybrida*) were inoculated with the sap of Siberian pea material, homogenized in 0.1 M sodium phosphate buffer pH 7.0 (2 : 1, w/v).

### Results and discussion

Spherical inclusions appear naturally during spring in the nucleus and cytoplasm of epidermal and parenchyma cells of Siberian pea shoots and flowers. These structures are more frequently present in epidermal and parenchyma cells of sepals, than in pedicels and petioles. Some phases have been detected where the bodies are characterized by their special size, form and cytochemical characteristics. At the time of buds' unfurling, there is, as a rule, one body in the nucleus. This structure is 1 µm in diameter, composed of closely packed fibrils and displays a direct connection with the nucleolus. The inclusion is more electron dense than the nucleolus and is not enclosed in a membrane. Later on, the intranuclear body increases in the diameter (Fig. 1) up to 5 µm.

During the process of the bodies' morphogenesis, changes of the nuclear membrane take place. The local enlargement of a perinuclear space occurs

at the firstly parallel-arranged membrane. Sometimes later, when the young leaves become middle-sized and flowers are unfurling, another body is observed in the cytoplasm. At the beginning, it adjoins to the external nuclear membrane (Fig. 2), which forms deep outgrowths in the cytoplasm, and displays numerous ribosomes. The further days, the cytoplasmic body increases in size up to 12  $\mu\text{m}$ . By the time of flowering, the intranuclear body may considerably exceed the nucleolus in size. The homogenous zone of lower electron density without the restrictive membrane and only with separate cisterns of the granular endoplasmic reticulum

surrounds the cytoplasmic body. By this time, the intranuclear body has disappeared. At the stage of normal-sized leaves and unfurled flowers, the homogenous zone increases in size, and its content starts crystallising. At this time, the cytoplasmic formation has a round or oval and often irregular angular form. Radial structures, consisting of orderly-arranged parallel fibrils, begin to form in the cytoplasmic body center and continue to grow in the homogenous zone (Fig. 3). By the end of flowering, the changes of the form of cytoplasmic inclusions take place. Their spherical centers disappear, and groups of independent orderly arranged fibrils are spread through the cytoplasm. Irreversible changes of inclusions persist. By the fruits' arrangement, cytoplasmic and intranuclear inclusions or their components are not seen in the cells.

Different cytochemical tests of Siberian pea epidermal and parenchyma inclusion-keeping cells were carried out to determine the possible composition of the cytoplasmic inclusions. After staining samples with Methyl green — Pyronin, these inclusions were red, and appeared violet after Azure B staining. Following the Feulgen procedure, only nuclei become bright red. These tests indicate the possible presence of RNA and absence of DNA in the structures. These cytoplasmic inclusions reacted positively with Fast green and Bromo phenol blue and became bright green and blue, respectively.

Our observations reveal that the intranuclear bodies share a number of morphological characteristics with the nucleolus, although the density of both types of bodies was not found to be equal. The presence of inclusions next to the nucleolus suggests the existence of some sort of relationship with the latter structure. It is reasonable to suggest that the inclusions originate from the nucleolus. It also appears likely that these structures are eventually secreted into the cytoplasm. This hypothesis derives support from the observed

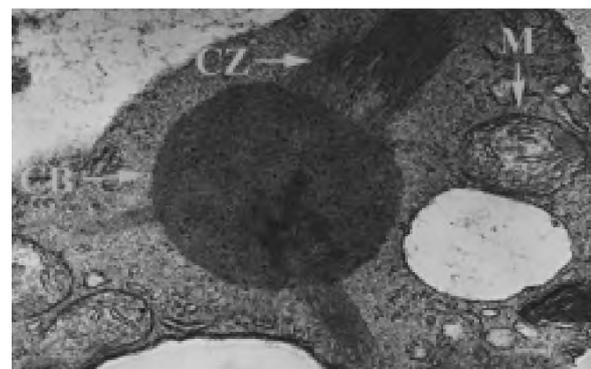
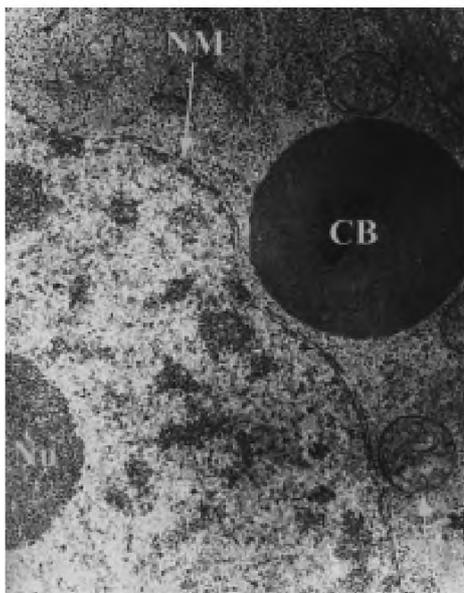
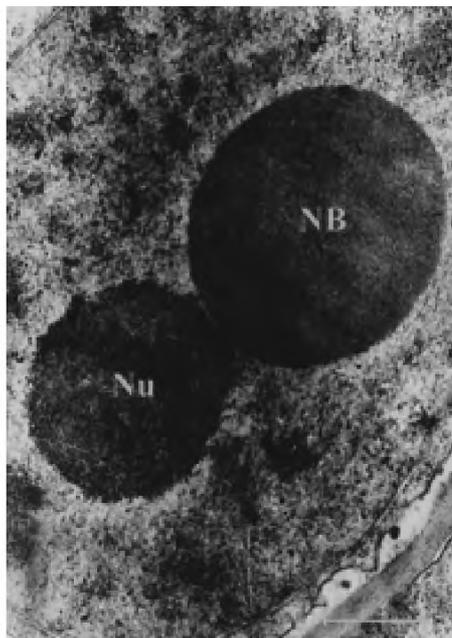


Fig. 1—3. Electron micrographs of portions of epidermal cells. 1. A nuclear body (NB) is attached to the nucleolus (Nu). 2. A cytoplasmic body (CB) located next to nuclear membrane (NM). 3. The process of crystallization of the homogenous zone: a cytoplasmic body (CB), a crystallization zone (CZ), and a mitochondrion (M). The bars = 1  $\mu\text{m}$ .

similarities between the intranuclear and cytoplasmic bodies. The latter ones are, indeed, frequently observed at the periphery of the nucleus but the fact that we have not seen these bodies in the process of migrating across the nuclear envelope most likely indicates that transport of the material takes place at the macromolecular level.

To determine the possible nature of the inclusions, indicator plants were inoculated with the sap of Siberian pea. At the occurrence of a virus infection, the artificial inoculation induces the appearance of symptoms in the indicator plants but

the infected plants did not show any symptoms. On the base of data of indicator plants' inoculation and because of the symptoms' absence on Siberian pea leaves and flowers, it is possible to conclude that the intranuclear and cytoplasmic bodies do not have a virus nature. These structures are, therefore, characteristics of healthy cells.

The localization of intranuclear and cytoplasmic inclusions mainly in cells of generative organs during spring indicates that their formation is associated with flowering. These inclusions appear to be composed of proteins and RNA.

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## ВНУТРІШНЬОЯДЕРНІ ТА ЦИТОПЛАЗМАТИЧНІ ВКЛЮЧЕННЯ В ЕПІДЕРМАЛЬНИХ ТА ПАРЕНХІМНИХ КЛІТИНАХ ЖОВТОЇ АКАЦІЇ

Ядра та цитоплазма епідермальних та паренхімних клітин, що ростуть, жовтої акації характеризується наявністю сферичних тілець. Ці структури є Фольген-негативними, але позитивно забарвлюються метиловим зеленим — піроніном та азуром В на РНК, а також міцним зеленим та бромфеноловим синім на основні білки. На ультраструктурному рівні тільця складаються з компактно упакованих фібрил та характеризуються як структури, що морфологічно змінюються. Ці включення не є вірус-індукованими, а притаманні нормальному здоровим клітинам.