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INTRACELLULAR CHANGES INDUCED BY THE DEFECTIVE KAZAKHSTAN STRAIN OF TOBACCO MOSAIC VIRUS

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Introduction

Goldin (1963) described the Kazakhstan strain (Ka) of tobacco mosaic virus (TMV) which differed from the type strain in many properties. On tobacco leaves the Ka strain caused light green or yellow orange spots which gradually increased and were transformed into rings. The symptoms spread very slowly to the upper parts of tobacco so that the top leaves were often without symptoms. On the leaves of *Nicotiana glutinosa, N. sylvestris* and *Datura stramonium* necrotic local lesions appeared which were very similar to the ones of many other tobamoviruses. Moreover, the Ka strain sometimes reacted positively with the serum against the type TMV. It is a characteristic of the Ka strain that its thermal inactivation point lies between 75° and 80°. In this property Ka distinctly differs from the type strain which is inactivated at 92°.

Brčák (1978) established that normal 300 nm long particles were very rare and very long particles were frequent in metal-shadowed sap preparations. He established that particles from dried leaves were shorter than 300 nm and were mostly not infective. Besides, in infected cells the hexagonal crystals were very rare and long coiled fibres very common.

On account of these properties Brčák (1978) considers that the Ka strain behaves similarly to defective strains of TMV.

Material and Methods

During this investigation the same Ka strain of TMV was used which Brčák (1978) had employed. This virus was conserved for a long time in dried leaves and periodically was cultivated in living specimens of Samsun tobacco.

Infected tobacco plants were employed for light and electron microscopy. The material for electron microscopy was fixed for 30 min in 1% (v/v) glutaraldehyde in cacodylate buffer and after appropriate washing in buffer was postfixed for 2 h in 1% (w/v) osmium tetroxide. After fixation samples of tissue were dehydrated in ethanol series and embedded in Araldite. The ultrathin sections were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiskop I.

Results

In tobacco plants infected with Ka strain the hexagonal crystals were infrequent but their presence indicated that sometimes normal TMV particles were formed. Elongated crystals similar to spikes were often present; they were fairly large and showed longitudinal striations (Fig. 1a). The intranuclear crystals often had also the form of a spike (Fig. 1b, d). Whether these crystals really are spikes or not, can only be proved by means of investigation of their fine structure. It is characteristic of spikes that their elongated virus particles are longitudinally orientated (Miličić 1977).

A well marked inclusion form is the long coiled fibres which build loops and figures in the form of number eight (Fig. 1e). The coiled fibres were very like the inclusions described first by Kassanis and Sheffield (1941) and studied later with regard to their fine structure by Wehrmeyer (1959). A similar form of coiled fibres is also present in the tobacco cells infected with the defective German PM2 mutant (Kassanis and Turner 1972) but it differs considerably in fine structure (Siegel et al. 1966).

We were specially interested in intranuclear crystals built by Ka strain (cf. Goldin 1963, Brčák 1978). Therefore we prepared some ultrathin sections of the nucleus. Figures 2 and 3 show that the part of the nucleus which contains the chromatin is placed in the periphery while in the middle of the nucleus is a mass which can assume a crystalline character. In some cases the mass shows an unclear structure which probably consists of irregularly curved fibrils (Fig. 2). It seems that for a while from this mass the stretched threads, which are parallelly arranged, can be differentiated (Fig. 3). The threads are about 90 Å wide.

It must be said that we had difficulties in preparing the intranuclear mass or crystals for investigation. In many cases the nuclear centre became empty, i. e. without the crystalline mass. Therefore, we succeeded in fixing only a part of the nuclei with intranuclear inclusions. We also had difficulties in staining the mass (stretched threads) with uranyl acetate: it was necessary to treat the mass 45 to 60 min to make the threads visible (Fig. 3).

Between two stretched threads there is an interspace which is not stainable and which alternates with the threads. It seems that the threads make the basic substance of the intranuclear crystals. Between

the regularly arranged threads and the peripheral nuclear part containing chromatin there are a lot of curved fibrils from which the regularly arranged threads have probably developed (Fig. 3).

The system of parallelly arranged and stretched threads is not present only in the nucleus but also in the cytoplasm. Fig. 4 shows that equal threads exist outside the nucleus. In the neighbouring cytoplasm the thin threads and the light interspaces alternate rather regularly (Fig. 4 ot).

While the threads in the nucleus stretch parallelly with the surface of the section, the cytoplasmic threads change the orientation during their course and run obliquely near their margin. In this last orientation it is visible that the stretched threads are rounded and their ends become darker grey (Fig. 4 ot). On the basis of figures described it is possible to suppose that the threads build at least a part of intranuclear and intracytoplasmic inclusions of Ka strain.

Discussion

Many defective TMV strains, e. g. Ni 118 and PM2, represent mutants obtained after treatment with nitrous acid (Kassanis and Milne 1971. Kassanis and Bastow 1971, Kassanis and Turner 1972). The defectivity of these mutants consists in their inability to form complete particles in changed exterior conditions. For instance, the strain Ni 118 builds hexagonal crystals and complete virus particles at 20° in infected cells. However, at 35° this virus builds neither hexagonal crystals nor complete particles, but it forms the viral RNA and insoluble coat protein in amorphous inclusion bodies.

The strain PM2, however, forms a defective protein which is not able at all to envelop its RNA. A variant of this strain, the German PM2, has a soluble protein, but the protein of the English PM2 variant is insoluble (Kassanis and Bastow 1971).

Brčák's opinion on the defective nature of the Ka strain was based partially on the fact that the particles rarely had a normal length of 300 nm. The particles derived from dried leaves were often shorter than 300 nm. Similarly, the defective nitrous mutants of TMV had particles often shorter than the normal ones, so e. g. the particles of Ni 118 were broken (Kassanis and Bastow 1971). Hariharasubramanian and Siegel (1969) found that the particle breaking of the mutant PM5 was caused by insufficiently tight packing of protein subunits. It seemed that the subunits were not always spirally packed but were arranged in the form of stacked disks in places where they broke.

The particles of the Ka strain were often very long in expressed sap, which was also an anomalous state (Brčák 1978). Other defective viruses sometimes have similar rather elongated particles. Kassanis and Woods (1968) reported that the partially purified strain RS produced elongated aggregates which were ten times longer than the normal particles but their width was normal, i. e. 18 nm. The RS strain is able to produce ring symptoms on tobacco leaves, resembling those caused by the Ka strain. Another defective strain, TC was thermoresistant and was therefore more infective at 35° than at 20°. This virus in purified preparations, which were kept at 20° many months, produced very long aggregates (Kassanis and Bostow, 1971, p. 167). These aggregates consisted only of protein.

Fig. 1. Hair cells of 'Samsun' tobacco leaves with inclusions of the Kazakhstan strain of TMV. (a) Elongated crystal with longitudinal striations. (b—d) Very long intranuclear inclusions which alter the form of nuclei. (e) Very long coiled fibres in the form of number eight. — Bar in figure (a) also relates to figures (b—d) and represents 300 μm. Bar in figure (e) represents 300 μm.

Sl. 1. Dlačne stanice listova duhana Samsun s inkluzijama kazahskog soja virusa mozaika duhana. (a) Produženi kristal s uzdužnim prugama, (b—d) intranuklearne inkluzije i (e) dugačka savijena vlakna u obliku broja osam. — Skala na sl. (a) vrijedi i za sl. (b—d) i iznosi 300 μm. Skala na sl. (e) iznosi 300 μm.

Fig. 2. Cross section of the nucleus of a tobacco cell. In the centre of figure the central nuclear part with irregularly curved fibrils is visible (cf). Around this part the nucleus periphery with chromatin is visible (ch). Bar marker represents 300 nm.

Sl. 2. Poprečni presjek kroz jezgru stanice duhana. Na sredini slike vidi se središnji dio jezgre u kojem se nalaze nepravilno savijene fibrile (cf). Oko toga dijela na rubu jezgre je kromatinska tvar (ch). Skala iznosi 300 nm.

Fig. 3. The nuclear part with chromatin (ch) is situated on the right. In the middle, stretched threads are differentiated (st) from curved fibrils. Only the peripheral part of the inclusion still contains curved fibrils (cf). Bar marker represents 300 nm.

Sl. 3. U perifernom dijelu jezgre nalazi se kromatinska tvar (ch). U sredini jezgre od savijenih fibrila diferencirale su se paralelno poredane opružene niti (st) dok je samo u perifernom dijelu inkluzije zaostao dio savijenih fibrila (cf). Skala iznosi 300 nm.

Fig. 4. In the upper part of figure nucleus with chromatin, curved fibrils and stretched threads. In the lower part intracytoplasmic stretched threads (st) are visible. The threads run partially parallelly with the surface of section (st). On the left they change the orientation and run obliquely; then they are visible sometimes in cross section (ot). Bar marker represents 300 nm.

Sl. 4. U gornjem dijelu slike vidi se dio jezgre sa savijenim fibrilama i opruženim nitima (st). U donjem dijelu slike vide se u citoplazmi opružene niti koje teku paralelno s ravninom presjeka (st), ali lijevo mijenjaju svoj smjer tako da se vide u poprečnom presjeku (ot). Skala iznosi 300 nm.

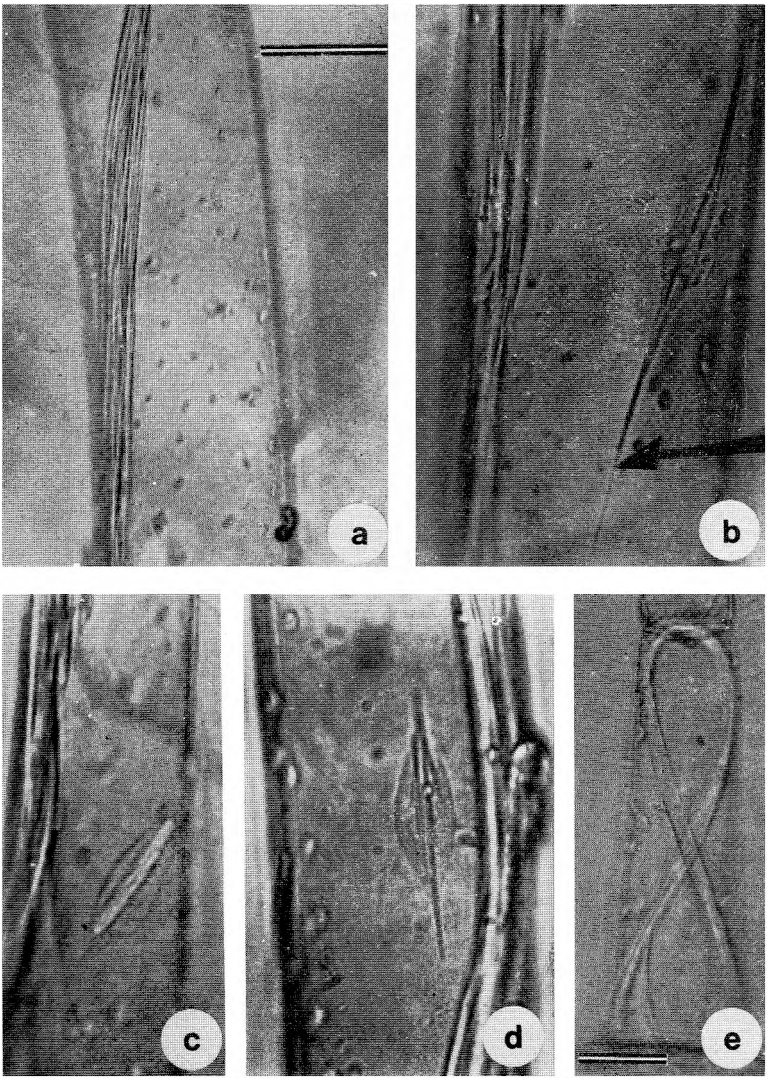

Fig. 1. — Sl. 1.

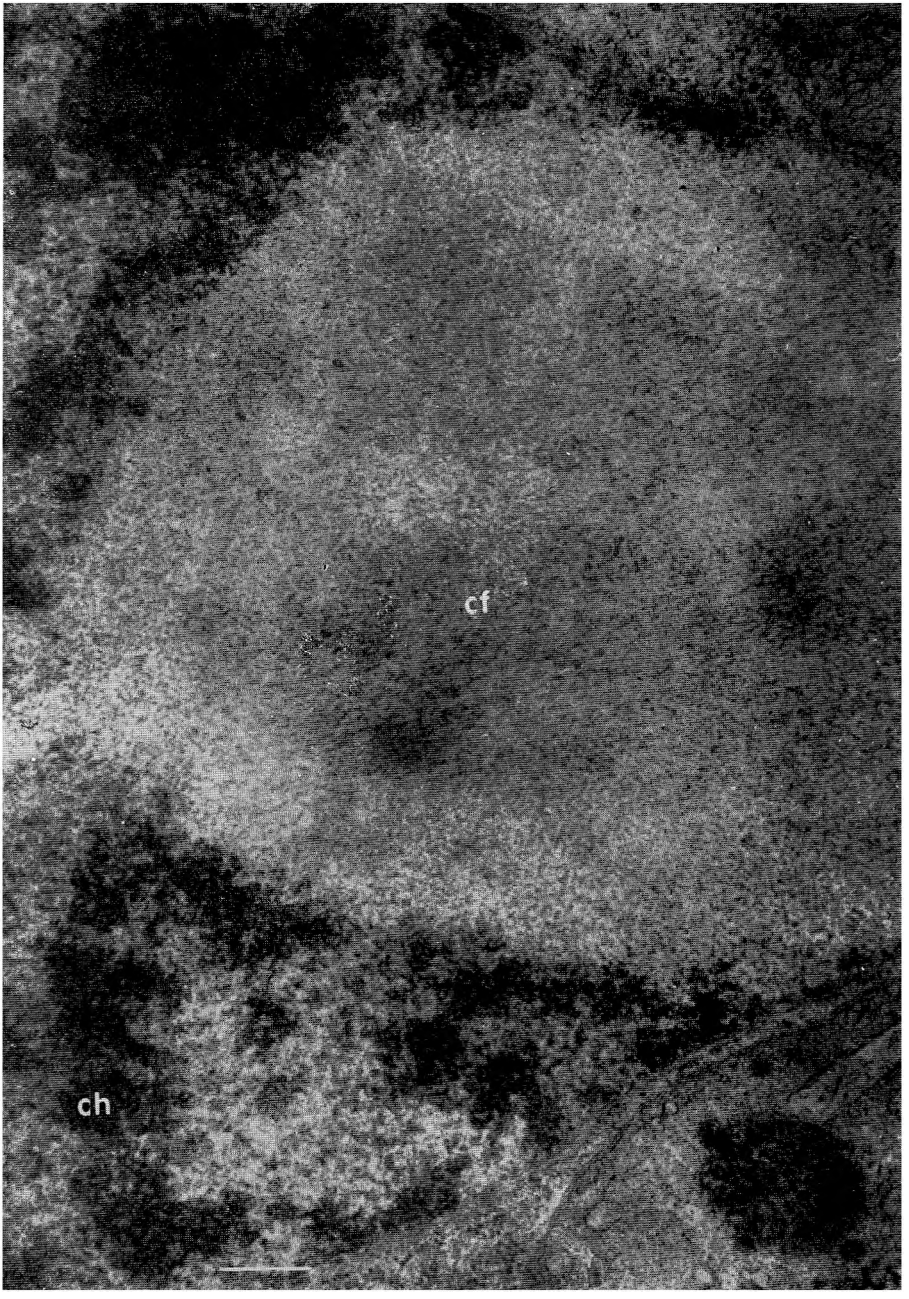

Fig. 2. — Sl. 2.

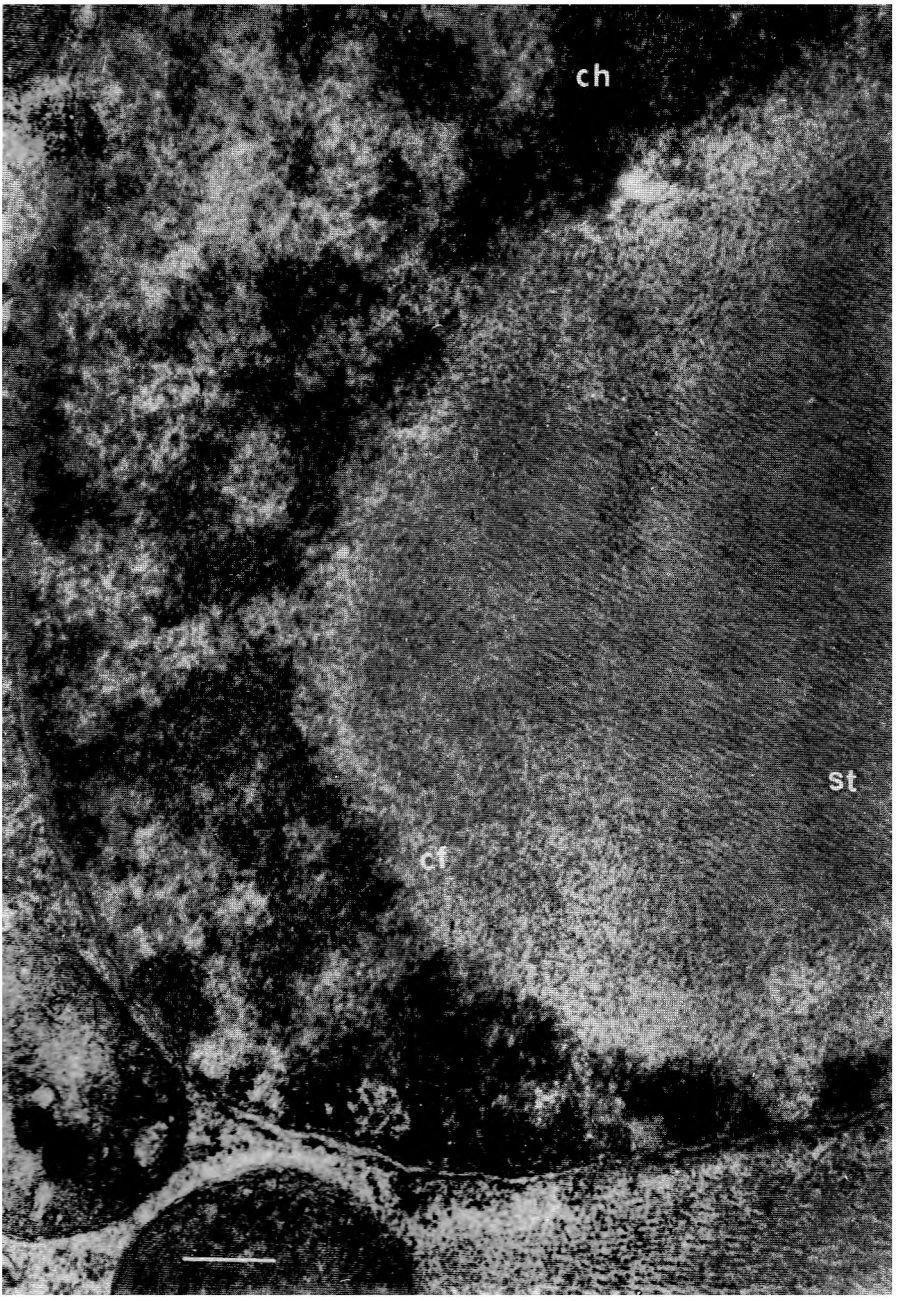

Fig. 3. — Sl. 3.

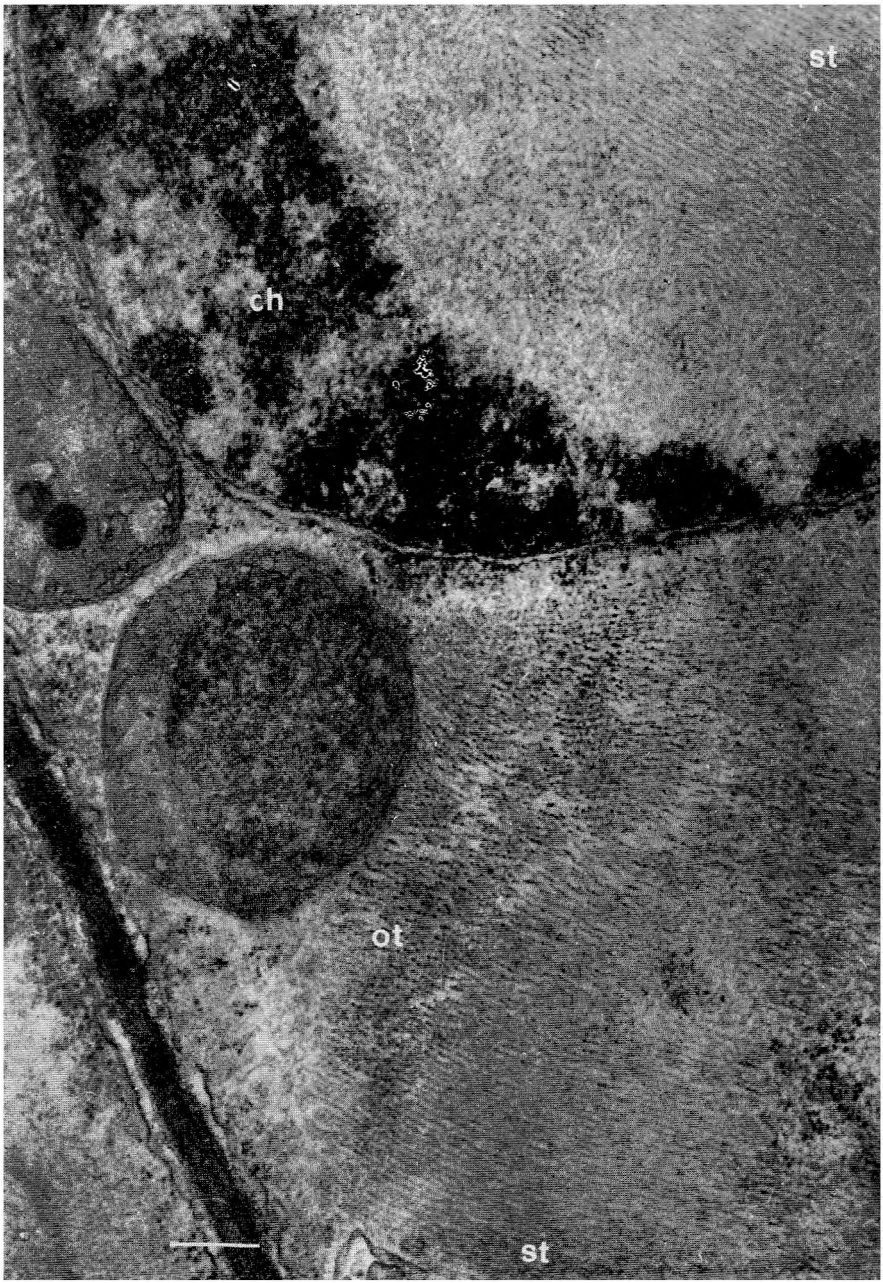

Fig. 4. — Sl. 4.

As the Ka strain is able to produce long coiled fibres, we shall discuss some problems in connection with this form of inclusion bodies. The coiled fibres of *flavum* strain investigated by Kassanis and Sheffield (1941) had complete particles (Kassanis and Turner 1972). Afterwards, Wehrmeyer (1959) studied the G2 yellow strain. His electron microscopic investigations of coiled fibres showed that the virus particles were spirally orientated and not longitudinally as in spikes. This spiral orientation of virus particles was confirmed also by investigation with polarisation microscope because the fibres never showed the positive birefringence in respect to their length which the spikes regularly did.

Wehrmeyer (1959) considered that the fibres were aggregates of fibrils. That these fibrils are built of a complete virus follows from the fact that they change their shape and transform into prisms after treatment with KSCN.

Long coiled fibres induced by the German PM2 strain have a special structure which was investigated by Zaitlin and Ferris (1964), and Siegel et al. (1966). The strain PM2 is not able to form complete virus particles but can multiply by means of its naked RNA. Its protein remains free and can first aggregate in open helical structures and then forms long coiled fibres. The open helical structures are different from tight packed and spirally arranged protein of common TMV. The helical protein has a diameter of 120 Å (Siegel et al. 1966).

Some elongated structures built of virus protein can arise in cells infected with common TMV. Kolehmainen et al. (1965) and Esau and Cronshaw (1967 a, b) described very long and parallelly arranged tubular components which were abundantly present in infected cells. According to these authors the tubular components are made of excess of coat protein (X-protein) which aggregates forming tubular protein bodies which do not contain RNA. The tubular components have a diameter from 191 to 283 Å.

The flexuous particles found by Kassanis and Turner (1971, Fig. 5) in plants infected with German PM2 are similar to the tubular components. Further like structures are the stretched threads which are described in this paper. The threads are very similar in shape to the flexuous particles. However, the width of threads and flexuous particles is different, i. e. 90 Å and 190 Å respectively. Besides, the flexuous particles have a hollow in their centre and the stretched threads are without a noticeable central cavity.

It would be interesting to investigate the chemistry of the stretched threads.

Summary

The Kazakhstan strain of TMV was described first by Goldin (1963). Later Brčák (1978) established that this virus resembled defective TMV strains, mainly on the basis of the presence of very long particles in the preparations made by dipping method.

This virus builds various types of crystalline inclusion bodies in cells, especially elongated crystals with striations and long coiled fibres (Fig. 1). Elongated crystals are similar to spikes and are present in the cytoplasm and in the nucleus.

In this study inclusion bodies were examined by electron microscope and it was found that the inclusions, at least sometimes, are not built of complete virus particles but of structures which are named stretched threads. These structures represent a kind of fibrils which are difficult to stain, have a diameter of about 90 Å and are parallelly arranged (Fig. 3 and 4). It would be important to solve the problem from which chemical material the stretched threads are built.

Literature

Brčák, J., 1978: Defective character of the Kazakhstan strain of tobacco mosaic virus. Plant Virology, Proc. 8th Conf. Czechslov. Plant Virologists.

Esau, K., and *J. Cronshaw*, 1967a: Relation of tobacco mosaic virus to the host cells. J. Cell Biol. *33* (3), 665—678.

Esau, K., and *J. Cronshaw*, 1967b: Tubular components in cells of healthy and tobacco mosaic virus-infected Nicotiana. Virology *33*, 26—35.

Goldin, M. I., 1963: Virusnye vključenija v rastitelnoj kletke i priroda virusov. Izdat. AN SSSR, Moskva.

Hariharasubramanian, V., and *A. Siegel*, 1969: Characterization of a new defective strain of TMV. Virology *37*, 203—208.

Kassanis, B., and *C. Bastow*, 1971: The relative concentration of infective intact virus and RNA of four strains of tobacco mosaic virus as influenced by temperature. J. gen. Virol. *11*, 157—170.

Kassanis, B., and *R. G. Milne*, 1971: An unusual inclusion in plants infected with a TMV mutant. J. gen. Virol. *11*, 193—195.

Kassanis, B., and *F. M. L. Sheffield*, 1941: Variations in the cytoplasmic inclusions induced by three strain of tobacco mosaic virus. Ann. appl. Biol. *36*, 9—17.

Kassanis, B., and *R. H. Turner*, 1972: Virus inclusions formed by the PM_2 mutant of TMV. J. gen. Virol. *14*, 119—122.

Kassanis, B., and *R. D. Woods*, 1969: Properties of some defective strains of tobacco mosaic virus and their behaviour as affected by inhibitors during storage in sap. Ann. appl. Biol. *64*, 213—224.

Kolehmainen, L., *H. Zech*, and *D. von Wettstein*, 1965: The structure of cells during tobacco mosaic virus reproduction. J. Cell Biol. *25* (2), 77—79.

Miličić, D., 1977: The structure and composition of crystalline inclusions of plant viruses. Acta Bot. Croat. *36*, 9—17.

Siegel, A., *G. J. Hills*, and *R. Markham*, 1966: In vitro and in vivo aggregation of the defective PM2 tobacco mosais virus protein. J. Mol. Biol. *19*, 140—144.

Wehrmeyer, W., 1959: Entwicklungsgeschichte, Morphologie und Struktur von Tabakmosaikvirus-Einschlußkörpern unter besonderer Berücksichtigung der fibrillären Formen. Protoplasma *51*, 165—196.

Wittmann, H. G., 1965: Die Proteinstruktur der Defektmutante PM2 des Tabakmosaikvirus. Z. Vererbungslehre *97*, 297—304.

Zaitlin, M., and *W. R. Ferris*, 1964: Unusual aggregation of an unfunctional tobacco mosaic virus protein. Science *143*, 1451—1452.

SAŽETAK

INTRACELULARNE PROMJENE IZAZVANE DEFEKTNIM KAZAHSKIM SOJEM VIRUSA MOZAIKA DUHANA

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Kazahski soj virusa mozaika duhana opisao je Goldin (1963). Poslije toga je Brčák (1978) ustanovio, naročito na osnovi nazočnosti vrlo dugačkih čestica u preparatima priređenim metodom uranjanja, da taj soj pripada skupini defektnih sojeva virusa mozaika duhana. Kazahski soj stvara u stanicama raznovrsne tipove kristaličnih inkluzija, osobito produžene kristale s uzdužnim prugama (sl. 1a) i dugačka savijena tjelešca (sl 1e). Produženi kristali bili su ponekad slični bodljikama (spikes), a bili su nazočni u citoplazmi i jezgri (sl. 1b, d).

U ovom radu istražili smo intracelularne inkluzije s pomoću elektronskog mikroskopa. Ustanovili smo da inkluzije barem ponekad nisu izgrađene od kompletnih virusnih čestica. U inficiranim stanicama zapazili smo strukture koje smo nazvali opruženim nitima i koje — kako se čini — izgrađuju neke tipove inkluzija. Strukture se sastoje od neke vrste fibrila koje se teško kontrastiraju, koje imaju dijametar oko 90 Å i paralelno su raspoređene. Čini se da su te niti izgrađene od virusnog materijala. Bilo bi važno kad bi se tijekom daljnjih istraživanja utvrdio kemizam toga materijala.

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