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Virus Specific Cytological Effects in Infected Plant Cells

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S u m m a r y

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Plant virus infections induce changes of host cell fine structures which comprize on the one hand pathological reactions of the plant cells - resulting from virus-induced disturbed balance of the host plant physiology - and on the other hand virus-specific cytological alterations directly associated to virus propagation. The latter are induced in infected cells of all host plants, irrespective whether they show external symptoms or 'latent' infections. Virus-specific alterations comprise two main categories: 1. Products of the viral propagation process are on the one hand virions in various types of arrangements in the infected cells including cellular inclusions of virion crystals. On the other hand viral non-structural proteins are expressed additional to the coat proteins which may form massive accumulations as cellular inclusions. 2. Various specifically structured alterations of the cellular membrane system occur which are either hypertrophied endoplasmic reticulum correlated with synthesis of viral proteins or ds-RNA containing vesicles possibly associated with nucleic acid replication. An overview over the manifold virus-specific cellular alterations is presented and discussed in respect of the protein composition of cellular inclusions and of the significance of observed membrane alterations for viral replication processes.

I n t r o d u c t i o n

Plant cells serve for an infecting virus as biochemical and molecular environment which can by the viral genome be determined to sustain the replication of the virus. This is achieved by the use of the host cells' protein synthesizing system for the production of non-structural proteins (NSP), including

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nucleic acid replicating enzymes, and the coat protein (CP) of the virus. The latter serves, together with viral nucleic acid (NA) molecules for the formation of new infective viral particles. The processes incited by a virus may disturb the biochemical balance of the host cells since host cell components including its energy conferring systems have to be used for the synthesis of viral components. These processes are in many virus-host systems not fully compatible with the host cells' physiological balance and, therefore, incite various symptoms of cellular degeneration, in the extreme cellular death.

Various external symptoms indirectly illustrate the complicated interactions between virus and host cells. They may occur in the whole host plant or may be localized on plant parts near the original infection sites. Virus symptoms may be specific to certain plant organs like the flowers (flower break symptoms) or to certain tissues like the phloem. Various distinct regular or irregular patterns of chlorotic or necrotic tissues develop on leaves indicating that the distribution of systemically spread virus is not really uniform throughout the plant. The effects of systemically spread virus are obviously regulated by more or less balanced interactions between the host metabolism, the virus and environmental factors. Hypersensitivity phenomena occur which lead to sudden cell death immediately upon the initial cell infection. These examples indicate highly complex interactions which have largely not been elucidated. The external symptoms are produced by infected tissues relatively late, at a time when viral propagation is already completed in the respective tissue. On the cellular level degeneration symptoms caused by the infection are observed e.g. in form of chloroplast senescence (lipid accumulation, vacuolization). External symptoms and alterations of cell fine structures which indicate reactions of the host cells to the disturbing influence of the virus are not further treated here.

The topic of this contribution are those alterations which appear to be essential for the virus and are induced early after infection in each host plant, even in latent infections. These alterations are considered as specific for virus species or genera or even families since they represent products of virus propagation or are associated with the process of their synthesis. Two main complexes of cytological observations can be classified: 1. cellular inclusions composed of products of the virus propagation like virions and viral NSP, and 2. virus-induced alterations of the host cells' membrane system. Virus specific cytological alterations of plant cells have since long time been studied morphologically and have been reviewed repeatedly (EDWARDSON & CHRISTIE 1977, MARTELLI & RUSSO 1977, FRANCKI & al. 1985, LESEMANN 1988). In recent time molecular biological tools have enabled more detailed analyses. Antibodies to expressed viral NSP proteins were used to localize viral proteins in cellular inclusions (MARTIN & al. 1992, RIEDEL 1998, RIEDEL & al. 1998) and nucleic acid probes to localize viral nucleic acids in suspected replication sites (RIEDEL 1998).

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Products of Virus Propagation

Virions in infected plant cells

Already in the last century large intracellular accumulations (several µm diameter) of proteinaceous material have been observed for the first time which are now known to be induced by certain virus infections. They could at that time not be correctly interpreted since plant viruses were not yet known. The inclusions were designated e.g. as protein spindles (in cacti) or crystalline inclusions (of tobacco mosaic virus (TMV)-infected tobacco). Improving light microscopy together with the developing discipline of plant virology, but especially the electron microscopy (EM) later allowed more detailed morphological analyses i.e. to distinguish between very diversely structured inclusions. By EM it could be recognized that many of the inclusions are composed of masses of aggregated, sometimes crystallized virus particles. It was also recognized that on the EM level the light microscopically visible inclusions are only one aspect of particle aggregates. Smaller aggregates or even loose aggregates or scattered particles may also exist. So a description of viral aggregates has now to base on EM.

The fine structure of the particle aggregates often appears as specific for the respective virus. Thus, particle aggregates in form of various crystalline arrays depend in their construction on particle shapes (LESEMANN 1988). Crystals of TMV show typical layers of rod-shaped particles with the particle ends in register. Filamentous particles of the genera Potex- or Carlavirus may form stacked plate aggregates of paracrystalline appearance. Spindle-shaped quasi-parallel arrangements have been recorded with cactus virus X, and filamentous particles of the *Potyviridae* may form monolayers of particles in close association with membranes of the tonoplast or of other organelles.

Isometric particles of tymo- or cucumo- or other virus genera may occur in hexagonal or cubic crystals or may form distinct tubular crystals of round or quadrangular shape, but may also occur in high concentration but scattered arrangement in cytoplasm and/or vacuoles and/or nuclei. Particles of +ssRNA viruses are primarily located in the cytoplasm and are only very rarely found within chloroplasts or mitochondria.

Particles of membrane-enveloped taxa like *Rhabdoviridae* or tospoviruses form arrangements in extra-cytoplasmic compartments, i.e. in the lumen of the endoplasmic reticulum (ER) or in the perinuclear space.

Accumulations of nonstructural viral proteins and viroplasms

NSP are needed for the nucleic acid replication, protein processing, factors enabling cell to cell movement or as helper components for vector transmission. Usually only small amounts may be needed and produced and then their occurrence can often not be documented as cytological structure. However, with some virus taxa, e.g. *Potyviridae,* or tobamoviruses, an overproduction of NSP occurs, what leads to the formation of conspicuous crystalline or amorphous cytoplasmic inclusions which probably are deposits of excess NSP and may contain single NSP or several together. Cylindrical inclusions (CI) of the *Potyviridae*, composed of CI

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protein only, are structures of complex morphology and specific for all members of the family. They may function for the cell to cell transport of *Potyviridae*. The compositions of NSP accumulations have recently been studied in detail for *Potyviridae* using antisera to individual NSP (RIEDEL 1998, RIEDEL & al. 1998).

Viruses with other genome organisations than +ssRNA viruses, like *Caulimo-, Rhabdo-, Reo- and Bunyaviridae* induce certain types of inclusions in the cytoplasm or in nuclei with granular or fibrillar matrices which contain components of the virus particles, immature particles and mature virus particles. Such inclusions are called viroplasms and are considered to be a kind of 'virus factories'.

Alterations of the Membrane System

Virus-specific membrane accumulations

Additional to virus particles and NSP, which are the cellular products of virus infections, the infected host cells produce several types of alterations of their membrane systems. They comprize with many virus genera more or less distinct proliferations of rough ER, but also more complex aggregates occur. Voluminous accumulations of hypertrophied ER result, e.g. with carlaviruses, potexviruses and others, which together with virus particles and host organelles form distinct cytoplasmic inclusions. Viral signals obviously cause the proliferation which might serve for virus induced increased protein synthesis. With members of the genus bymovirus, an extreme degree of hypertrophy of ER elements occurs which is characterized by conspicuous three-dimensional crystal-like arrays of densely aggregated membranes (coat of mail-structure) (LESEMANN 1988).

Membrane associated vesicles

Two categories of virus-specific ds-RNA containing vesicles are induced in cells infected by +ssRNA viruses (LESEMANN 1991). The vesicle types apparently are correlated to the two main supergroups of +ssRNA viruses as defined on the basis of their principal genome organizations, picorna-like and sindbis-like viruses (GOLDBACH 1986).

Picorna-like plant viruses like *Potyviridae* and *Comoviridae* induce clusters of free vesicles in the cytoplasm which appear to contain viral dsRNA and which derive from buds of ER-like membranes.

In contrast, the sindbis-like viruses induce membrane-associated vesicles which are formed at the membranes of various organelles. Flask-shaped vesicles c. 100 nm in diameter and containing viral dsRNA are budding from the cytoplasmic side into the adjacent extra-cytoplasmic compartments. Such vesicles are formed at sites specific for the inducing virus or genus at membranes of chloroplasts, mitochondria, peroxisomes, the ER, the nuclear envelope or the tonoplast. The vesicles are likely to represent the morphological equivalent of the functionally already detected viral membraneous replicative complexes (LESEMANN 1991, RIEDEL 1998).

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Conclusions

The above short review can only give a rough description of the diverse cytological effects which may be understood as indicators for the underlying biochemical and physiological events started in a host cell by a virus infection. The diversity of phenomena illustrates how complicated and broad the field of virus-specific effects is. Even more complex may be the field of tertiary and higher grades of plant reactions which eventually show up as externally visible symptoms. Many observations indicate that the physiological status of the host plant (temperatures, light regimes, hormones) influences virus infections e.g. in respect of propagation rates or degree of virus spread into meristematic tissues. In fact some influences are known in the field of hormone actions. But up to now causal reaction chains need much further elucidation.

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Artikel/Article: Virus Specific Cytological Effect in Infected Plant Cells. 41-45