한국현미경학회지 제41권 제4호 2011
Korean J. Microscopy 41(4), 229~234(2011)

종 설

Occurrence of Nuclear Inclusions in Plant Cells

InSun Kim*

Biology Department, College of Natural Sciences, Keimyung University, Daegu 704-701, Korea
(Received December 14, 2011; Revised December 23, 2011; Accepted December 26, 2011)

식물세포 내 핵 함유구조 발달 양상

김 인 선*

계명대학교 자연과학대학 생물학과

ABSTRACT

The occurrence of nuclear inclusions has been reported in various plant groups from primitive ferns to higher flowering plants. Their presence within a group seems to be randomly distributed without any phylogenetic relationships among species. According to the current survey, nuclear inclusions have been widely documented in more than several hundreds of species from various families of plants. The morphology and internal structures of nuclear inclusions are diverse and at least five types of inclusions develop within plant nuclei; amorphous, crystalline, fibrous, lamellar, and tubular form. Among these types, crystalline inclusions are the ones that are the most frequently reported. The inclusions are not bound by membranes and appear to be related to the nucleoli, either spatially by a close association or by an inverse relationship in size during development. The idea that nuclear inclusions are of a proteinaceous nature has been widely accepted. Further link to nucleolar activity as a protein storing site has also been suggested based on the association between the nucleolus and nuclear inclusions. Various investigations of nuclear inclusions have revealed more information about their structural features, but characterizing their precise function and subunit complexity employing molecular analysis and 3-D reconstruction remains to be elucidated. Tilting and tomography of serial sections with appropriate image processing can provide valuable information on their subunit(s). The present review summarizes discussion about different nuclear inclusions in plants from previous works, giving special attention to their fine, ultrastructural morphology, function, and origin.

Keywords : Crystalline structures, Nuclear inclusions, Plant cells, Proteinaceous nature

INTRODUCTION

A wide variety of subcellular inclusions have frequently been reported to occur within plant or animal cells. They are located within various cellular organelles, such as the nucleus, plastid, mitochondria, endoplasmic reticulum, or microbody (Bosabalidis & Papadopoulos, 1983; Gunning & Steer, 1995; Chernyshev, 2000). They can also occur freely in the cytoplasm. Except for when inclusions are formed in plastids (Kim, 2009), the nucleus is usually the organelle that exhibits several types of inclusions within a cell.

The author would like to thank Dr. Kazuyoshi Murata of the National Institute of Physiological Sciences (NIPS), Okazaki, Japan for providing resources, including the H-1250M HVEM, and for technical assistance during the preliminary collaborative investigation of inclusions in plant species.
* Correspondence should be addressed to Dr. InSun Kim, Biology Department, College of Natural Sciences, Keimyung University, Daegu 704-701, Korea. Ph.: (053) 580-5305, Fax: (053) 580-5305, E-mail: botany@kmu.ac.kr

The nucleus is the most prominent compartment of plant or animal cells with a highly organized dynamic structure that contains well-defined, sub-organelle regions (Gonzalez-Melendi et al., 2000). Various types of nuclear inclusions (NIs) occur widely in both plant and animal cells; however, the size of the nucleoli (Kato, 2003) as well as composition and function of the nucleus differ considerably between plant and animal cells (Rose et al., 2004).

The formation of NIs in plants is a relatively widespread phenomenon, which has been reported in at least a couple hundreds of species as early as in 1970 (Wergin et al., 1970). The NIs have been documented from primitive species of ferns to gymnosperms and flowering plants, including carnivorous plants (Thomas & Gouranton, 1979; Vintejoux, 1984). Other features concerning shape, size, and number of Nis per nucleus have been used and emphasized for taxonomic relevance in some plant taxa (Eleftheriou & Kristen, 1988). Earlier investigation of inclusion bodies in more than 80 different species has revealed their presence in the nucleus (Septa, 1979). Furthermore, systematic affinities among surveyed species within a certain family have been discussed, although the study's data and conclusion seem somewhat questionable due to poor results based on simple line drawings (Septa, 1979).

Several forms of inclusions have been found within nuclei of cells and tissues from leaf, stem, root, flower parts, and even from embryo (Villiers, 1968) or pollen mother cell (Benett et al., 1979). In some plants, the size of nuclei can differ even within the same tissue. Nuclei from shoot apical meristems and young leaves of *Hedera* have indicated that central zone nuclei are larger and less dense than peripheral zone nuclei within different growth forms (Polito & Chang, 1984). A study of the structure and behavior of inclusions within the nuclei of root apical meristem in *Dryopteris* during mitosis has described changes that occur during cell division (Burgess, 1971). The number of inclusions per nucleus and changes in inclusion numbers decline as the tissue matures.

Studies using electron microscopy have confirmed the presence of inclusion bodies in both normal tissues and infected, or neoplastic, tissues. The ability of viruses to induce NIs has also been documented ultrastructurally (Langenberg, 1982; Langenberg & Zhang, 1997; Silvestro & Chapman, 2004; Reunov et al., 2006). However, evidence gathered from many studies strongly support the view that the occurrence of NIs in plants are not viral in nature in most cases (Wergin et al., 1970; Ciampolini et al., 1980; Kim, unpublished), even though NIs have been observed in fasciated stem cells of *Forsythia*. The NIs in *Forsythia* regularly form internal sub-structures with periodic arrangements (Codaccioni, 1973). Hence, the possibility of NI induction by viruses has been excluded from this article: data collected for the present study is based on normal, completely virus-free plants. Another type of dynamic, subnuclear structures called speckles, known to be located in the interchromatin regions of animal cells (Lamond & Spector, 2003), have not been included since they are rare and have only been documented recently in plant cells (Yu et al., 2009). The aim of this review is to summarize current knowledge on the occurrence of NIs in plants and ultimately facilitate a better understanding of the plant nucleus.

NUCLEAR INCLUSIONS

1. Types and Sub-structures

Nuclear inclusion is a term that refers to any discrete structure found in the nucleus that does not appear to be nucleolar or heterochromatic in nature (Wergin et al., 1970). The inclusion has no limiting membranes, and in many cases, they are the largest and most conspicuous structures within the nucleus. Frequently, they are larger than the nucleolus. Many occupy a large portion, up to 40%, of the total nuclear cross-sectional area (Eleftheriou, 1987). The growth of large, long inclusions can cause deformation of the nuclear membrane and thus alteration in nuclear shape, although this rarely occurs (Wergin et al., 1970). The size, shape, and arrangement of inclusions among various plant species have been investigated by numerous researchers. The differences documented in such studies are as follows.

The size and structural features of NIs are quite diverse. In studies that have comparatively investigated the fine structure of NIs in a variety of plant species, several distinct types have been recognized depending on their ultrastructural morphology. They have sub-structures or subunits of amorphous, crystalline, fibrous, lamellar, paracrystalline, tubular nature, striated leaflets (Wergin et al., 1970; Eleftheriou & Kristen, 1988), or even polyhedral forms (Burgess, 1971). Two or more types of inclusions may coexist within the same nucleus; nevertheless, the relationship between these different types is unclear. A possibility of simply representing different stages of differentiation has been suggested for such coexistence in the same investigation, while no indication of ontogenetical association has been obtained (Wergin et al., 1970). In species with two to four types of inclusions, they are commonly found in the same nucleus,

but the size of inclusions may vary with particular stage of cell development (Wergin et al., 1970). Another example can be found in NIs of *Scutellaria* (Thaler & Ameluxen, 1985; Thaler & Gailhofer, 1985, 1988), where two types have been identified particularly in glandular hairs; lamellae of 12 nm and tubules of 45 nm in diameter (Thaler & Gailhofer, 1988). Within these types, globular particles have also been found in the tubules and an electron dense filament in the channel.

A highly ordered, repeating pattern of inclusions has been recognized continuously in a large number of examined species. The epidermis of *Linaria* ovaries and leaves of *Incarvillea* consist of highly ordered tubules with a diameter of about 20 nm (Dudek & Hesse, 1980). A similar tubular pattern of NIs in leaves and styles of *Linaria* has been noted (Ciampolinietal, 1980). In leaves and style tissues of *Linaria*, inclusions are composed of layers of tubules spaced 9～10 nm apart. Within each layer, tubular direction is offset 60° with neighboring tubular layers. In such highly ordered structures of regularly arranged tubules, a diameter of about 23～26 nm has been noticed with the same appearance as cytoplasmic microtubules (Ciampolini et al., 1980). Similar to inclusions of *Linaria* tissues, stacks of lamellae with spacing of about 10.5 nm between sub-structures have been frequently observed within the nuclei of *Salvia* glandular hairs (Schnepf, 1971). In crystalline substructured inclusions, NIs usually exhibit a highly ordered periodicity as well-demonstrated in *Olea* leaf cells (Eleftheriou, 1987). In this respect, the NIs are quite similar to crystalline sub-structures encountered in plastids (Kim, 2006, 2006a, 2009; Lee, 2007) or in the cytoplasm (Bosabalidos & Papadopoulos, 1983; Kim, unpublished). At high magnifications, the inclusions reveal a sub-structure of crystalline order either with paralleled rows or with intersected subunits having a highly ordered periodicity of approximately 10 nm. As many as 10 crystal bodies have been found in each inclusion in Olea sections that have been examined, although four or five inclusions have been commonly shown to exist within the meristem cell nuclei (Eleftheriou & Kristen, 1988).

2. Correlation with Nucleolus, Properties, and Functions

Correlation between NI and the nucleolus has particularly drawn much attention as researchers continue to investigate differences in size, shape, and arrangement of NIs in detail in different species. Through histochemical and/or enzymatic digestion analysis, their proteinaceous nature has been ascertained since the early 1970's (Wergin et al., 1970; Raju, 1982). Successful digestion of the tubules with pepsin in gland hairs of *Scutellaria* clearly indicates their proteinaceous nature (Thaler & Gailhofer, 1988).

A study concerning the structure and behavior during mitosis has found decreases in NI numbers within the nucleus and the number of such inclusions per nucleus in *Dryopteris* root tissue maturation (Burgess, 1971). From this study, the NIs of a cubic lattice exhibited spacing between subunits of approximately 10 nm. In *Dryopteris* cell division, NIs have been found in chromosomes immediately after nuclear membrane breakdown, but they reformat early interphase stages by crystallization of an amorphous material within the nucleoplasm. Elongated NIs with lamellar organization occurs in mesophyll cells mostly attached to the nucleoli in *Scutellaria* (Thaler & Gailhofer, 1985) and a similar arrangement of the lamellae has also been attained in *Pinguicula* (Thomas & Gouranton, 1979). A large but different type of nuclear inclusion bodies, about 250 nm in diameter and consisting entirely of acidic protein, have been analyzed in the nuclei of maturing egg cells of *Pteridium* gametophyte (Bell, 1983). In the archegoniate region of this fern specimen, the NI proteins represent nuclear structural components that are experiencing temporary displacement and aggregation of nuclear material. In addition, a consequence of the fine chromatin dispersal has been proposed as another possible role.

In many cases, a close association to the nucleolus has been found, and often these inclusions appear to have proximal associations that may correlate with the multi-functional or unexpected capabilities of the nucleolus (Olson et al., 2000; Boisvert et al., 2007). The nucleolus and the NI appear to be related, either physically through proximity or through an inverse size relationship during development. Close proximity of inclusion to nucleolus, or actual contact between the two structures, has been noted repeatedly in many flowering plants. In fact, the inverse size relationship between NI and the nucleolus has been most strikingly documented in developmental studies of a fern species. Similar inverse size relationship between the two has been indicated by observations of other fern and flowering species (Wergin et al., 1970). Contrary to this, however, no specific association between NI and the nucleolus has been proposed in other species (Burgess, 1971).

Coincidentally, the onset of differentiation and subsequent cell division has been noted with the appearance of NIs (Wergin, 1970). The view that NIs play a possible role in reserving or storing forms of protein is widely accepted. Presumably, the protein is synthesized in the nucleoli and may be used when the plant suffers from malnutrition. However, in *Olea* species,

the NIs are persistent throughout seasons and are found in young and mature fruit (Eleftheriou & Kristen, 1988). It has been suggested that NIs are related to nucleolar activity probably as a storing site of proteins (Ciampolini et al., 1980). It is generally agreed that environmental conditions may not be responsible for the occurrence of NIs in plants (Eleftheriou & Kristen, 1988). Numerous sizable crystalline NIs, presenting glycoprotein molecules consisting of proteins and oligo- or polysaccharides, have been revealed during ultrastructural and cytochemical examination of *Olea* leaf segment (Eleftheriou & Kristen, 1988). Furthermore, differences in the high density subunit arrangement within those NIs have been established. Another speculated role concerning the formation of the NIs is in stylar cells of *Olea* flowers. In these flowers, NIs have been found after the tissue ceases its function: they seem to be connected to senescence (Eleftheriou & Kristen, 1988).

It is clear that in adventitious roots of *Dryopteris* NIs are a common feature of the meristem and that subsequent divisions and differentiation have been accompanied by a decline of the number of inclusions (Burgess, 1971). The cycle of nuclear division serves to eliminate NIs from the nucleoplasm. In late telophase after completion of envelopment, daughter nuclei have never been found to contain NIs. It is believed that there exists some mechanisms for removing and restoring NIs in the nucleus, although the cytoplasm only rarely contains such inclusions (Burgess, 1971). It seems likely that the cytoplasmic crystals are totally broken down during early interphase, and new subunit material is synthesized at the same time, or at least made available for the formation of crystals within the nucleus (Burgess, 1971).

FUTURE RESEARCH

The occurrence of the inclusion within the nucleus has been investigated mostly in the tissues of the interphase stage where the structure and properties may be limited. A comprehensive examination of NIs during development, from the initial stage to the senescence, may be necessary to reveal their authentic attributes within nuclei and the cell itself. Progress in NI research at the ultrastructural level and in immunocytochemistry will definitely provide important information concerning their precise function and sub-structural complexity. Advanced future works with the improved techniques and accumulation of knowledge about nuclear metabolism will further disclose the functional significance of these structures. Correlations between the presence and function of inclusions during nuclear development and differentiation can be achieved with comprehensive investigations. The need to develop experimental techniques for the isolation of NI and identification of the internal subunits has been emphasized lately.

This review summarizes our current knowledge about the occurrence of NIs in various plants from ferns to flowering plants. Compared to inclusions in chloroplasts and other cellular organelles, our understanding of NIs in plants is still in the infant stage. Isolation of NIs from plant cells can make chemi-

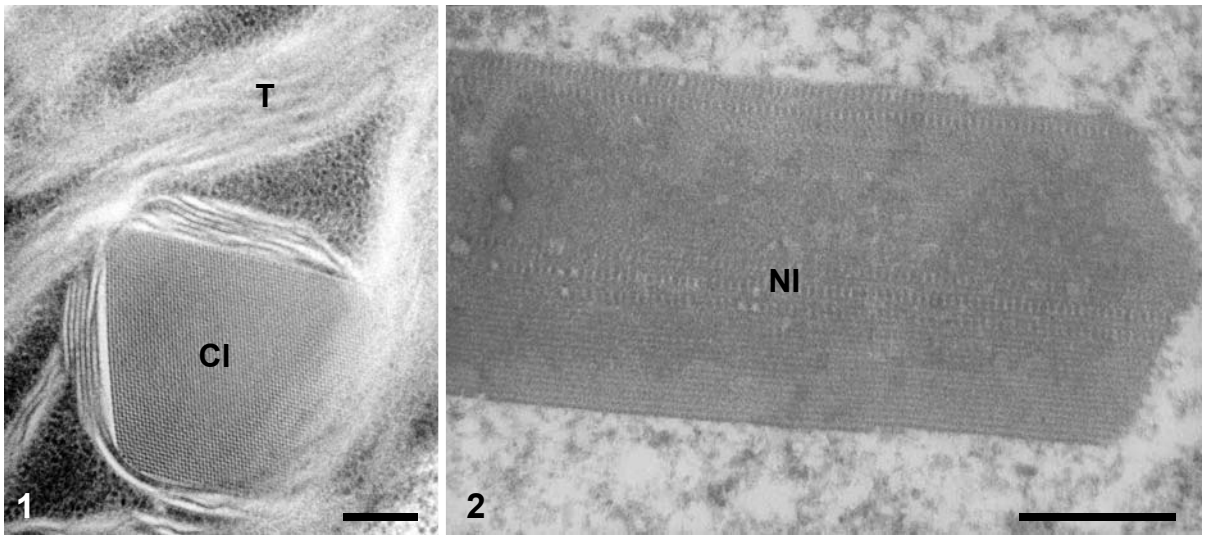

Fig. 1. The crystalline inclusion (CI) formed in the mesophyll chloroplast of *Salsola* leaf. T, thylakoidal membrane. HVEM. Bar=0.8 μm.
Fig. 2. Part of the nuclear inclusion (NI) found within cortical cells of the *Salvinia* leaf. Bar=200 nm.

cal characterization of the inclusions possible, and this in turn may shed new light on nucleolar and nuclear metabolism.

Extensive investigation of NIs in molecular research along with in the 3-D reconstruction field will further illuminate a blueprint for building a better morphology and ultrastructure of NIs at the sub-structural level. The need for characterizing the 3-D structure of subunits from serial sections is great as pointed out in recent plastid studies (Kim, 2006, 2006a, 2009). Application of high voltage electron microscopy (HVEM) with appropriate image processing is expected to improve analysis of 3-D subcellular structures of the inclusions since thick sections, up to several microns, of tissue samples can be examined by HVEM. By employing these methods, the periodicity of inclusion subunits can be assessed as in the case of plastids (Kim, 2006a, 2009, Fig. 1). In a preliminary examination of Nis in water fern *Salvinia*, a peculiar mode of formation has been revealed recently (Kim, unpublished, Fig. 2). This implies that applications of HVEM may allow the reconstruction of 3-D NIs, which will lead to an enhanced resolution of the internal sub-structures and a more in-depth analysis of NIs.

REFERENCES

Bell PR: Nuclear bodies in the maturing egg cell of a fern, *Pteridium aquilinum*. J Cell Sci 60 : 109-116, 1983.

Benett MD, Smith JB, Simpson S, Wells B: Intranuclear fibrillar material in cereal pollen mother cells. Chromosoma 71 : 289-332, 1979.

Boisvert FM, van Koningsbruggen S, Navascues J, Lamond AI: The multifunctional nucleolus. Nat Rev Mol Cell Biol 8 : 574-585, 2007.

Bosabalidis AM, Papadopoulos D: Ultrastructure, organization and cytochemistry of cytoplasmic crystalline inclusions in *Organum dictamnus* L. leaf chlorenchyma cells. J Cell Sci 64 : 231-244, 1983.

Burgess J: The structure and mitotic behaviour of a nuclear inclusion in a fern. Planta 96 : 238-247, 1971.

Chernyshev DP: Structure and possible nature of nuclear and cytoplasmic inclusions in plant cells. Tsitologiia 34 : 65-76, 2000.

Ciampolini F, Cresti M, Dominicis V De, Garavito RM, Sarfatti G: Intranuclear crystalloids in leaves and styles of *Linaria vulgaris* Mill. J Ultrastruct Res 71 : 14-21, 1980.

Codaccioni M: Description d'inclusions intranucléaires dans les cellules des tiges fasciées des *Forsythia* sp. Protoplasma 76 : 283-287, 1973.

Dudek A, Hesse M: Tubuläre intranucleäre Einschluβkörper bei *Linaria vulgaris* L. Alpina (Scrophulariaceae) und *Incarvillea variabilis* (Bigoniaceae). Pl Syst Evol 135 : 127-135, 1980.

Eleftheriou EP: A comparative study of the leaf anatomy of olive trees growing tin the city and the country. Environ Exp Bot 27 : 105-117, 1987.

Eleftheriou EP, Kristen U: Ultrastructural and cytochemical examination of the nuclear crystalloid inclusions of *Olea europaea* L. mesophyll cells. Trees 2 : 261-226, 1988.

Gonzalez-Melendi P, Beven A, Boudonck K, Abranches R, Wells B, Dolan L, Shaw P: The nucleus: a highly organized but dynamic structure. J Microsc 198 : 199-207, 2000.

Gunning BES, Steer MW: Plant Cell Biology. Jones and Bartlett Publishers, Boston, 1995.

Kato N: Structural analysis of living plant nuclei. Genet Engineer Principle Meth 25 : 65-90, 2003.

Kim I: Changes in the plastid ultrastructure during *Sedum rotundifolium* leaf development. J Plant Biol 49 : 376-383, 2006.

Kim I: High voltage electron microscopy of structural patterns of plastid crystalline bodies in *Sedum rotundifolium*. Kor J Electron Microsc 36 : 73-82, 2006a.

Kim I: A survey of plastid crystals and microtubules in flowering plants. Kor J Microsc 39 : 73-80, 2009.

Lamond AI, Spector DL: Nuclear speckles: a model for nuclear organelles. Nat Rev Mol Cell Biol 4 : 605-612, 2003.

Langerberg WG: Fixation of plant virus inclusions under conditions designed for freeze-fracture. J Ultrastruct Res 81 : 184-188, 1982.

Langerberg WG, Zhang L: Immunocytology shows the presence of tobacco etch virus P3 protein in nuclear inclusions. J Struct Biol 118 : 243-247, 1997.

Lee KB: Ultrastructure of crystalline inclusion in the thylakoids of dodder (*Cuscuta japonica*) plastids. J Plant Biol 50 : 325-330, 2007.

Olsen MOJ, Dundr M, Szebeni A: The nucleolus: An old factory with unexpected capabilities. Trends Cell Biol 10 : 186-196, 2000.

Polito VS, Chang Y: Quantitative nuclear cytology of English ivy (*Hedera helix* L.). Pl Sci Letters 34 : 369-377, 1984.

Raju CR: A staining method for light microscopic study of nucleolar structure in plant cells. Stain Tech 72 : 55-60, 1982.

Reunov AV, Lapshina LA, Nagorskaia VP, Poliakova Am, Lega SN: Ultrastructure of parenchymal leaf cells of different soybean varieties systematically infected with soybean mosaic virus. Tsitologiia 48 : 208-215, 2006.

Rose A, Patel S, Meier I: The plant nuclear envelope. Planta 218 : 327-336, 2004.

Schnepf E: Die Feinstruktur der lamellären Einschluβkörper im Zellkern und im Cytoplasma der Drüsenhaare von *Salvia glutinosa*. Protoplasma 73 : 67-72, 1971.

Silvestro SR, Chapman GB: A transmission electron microscope study of "New Dawn" climber rose (*Rosa wichuraiana* × *safrano*) exhibiting rose rosette disease. Plant Cell Rep 23 : 345-351, 2004.

Septa F: Weitere untersuchungen über proteinkörper in Zellkernen und ihre taxonomische Bedeutung. Pl Syst Evol 132 : 1-26, 1979.

Thaler I, Ameluxen F: Einschuβkristalle und Vacuolenkörper in den Zellen von *Scutellaria altissima*. Phyton 25 : 309-316, 1985.

Thaler I, Gailhofer M: Einschuβkörper in den Zellen von *Scutellaria altissima*. Phyton 25 : 309-316, 1985.

Thaler I, Gailhohofer M: Zwei verschiedene Zellkerneinschlüsse in den Drüsenhaaren von *Scutellaria altissima* L. Phyton 28 : 249-259, 1988.

Thomas D, Gouranton J: Ulttastructuraal and autoradiographic study of intranuclear inclusions of *Pinguicula lusitanica* L. Planta 145 : 89-93, 1979.

Villiers TA: Intranuclear crystals in plant embryo cells. Planta 78 : 11-16, 1968.

Vintejoux C: Inclusions intranucléaires *d'Utricularia neglecta* L. (Lentibulariacées). Ann Sci Nat Bot 6 : 203-205, 1984.

Wergin WP, Gruber PJ, Newcomb EH: Fine structural investigation of nuclear inclusions in plants. J Ultrastruct Res 30 : 533-557, 1970.

Yu X, Sayegh R, Maymon M, Warpeha K, Klejnot J, Yang H, Huang J, Lee J, Kaufman L, Lin C: Formation of nuclear bodies of *Arabidopsis* CRY2 in response to blue light is associated with its blue light-dependent degradation. Plant Cell 21 : 118-130, 2009.

＜국문초록＞

식물세포 핵 내에 형성되는 여러 형태의 함유구조 특성 및 발달양상을 다양한 식물에서 조사하여 비교 논의하였다. 이들은 원시적인 양치식물에서부터 고도로 분화한 현화식물에 이르기까지 많은 식물군에서 발달하나, 종간에는 계통적학인 유연관계 없이 각각 독립적으로 나타난다. 핵 내 함유구조는 내부 미세구조 특징에 따라 부정형(amorphous), 결정형(crystalline), 섬유상(fibrillar), 판상(lamellar), 미세관상(tubular)의 5～6 유형으로 구분된다. 가장 흔한 유형은 결정형으로 수 마이크론에 이르는 비교적 커다란 구조를 이루며, 내부구조 간 격자거리는 약 10 nm로 배열되어 있다. 핵 함유구조들은 막으로 둘러싸이지 않고 막과는 무관하게 발달하며, 핵 기질 내 인과의 구조적, 기능적 연계성이 강하게 추정되고 있다. 핵 결정체 및 미세구조 형성에 대한 연구는 이들의 구조와 기능에 대하여 새로운 정보를 제공하나, 핵 결정체에 대한 3차원적 입체구조 분석과 이들 구조를 핵에서 분리시켜 연구할 분자생물학적, 생화학적인 실험법들이 요구되고 있다. 특히, 핵 결정의 3-D 입체구조를 재구현할 수 있는 HVEM 고압전자현미경 및 tomography에 의한 연속절편 연구는 이들 구조에 대한 심도 있는 정보를 제공할 것이다. 본 논문에서는 다양한 식물세포 내 핵에 발달하는 함유구조에 대하여 연구된 구조 및 기능 등을 연계하여 그 중요성을 종합적으로 논의하였다.