A LIGHT AND ELECTRON MICROSCOPE STUDY
OF SMALL, SPHERICAL NUCLEAR BODIES
IN MERISTEMATIC CELLS OF ALLIUM
CEPA, Vicia Faba, AND RAPHANUS SATIVUS

J. G. LAFONTAINE, Ph.D.
From the Department of Biology, Laval University, Quebec, Canada

ABSTRACT
Interphase, preprophase, and prophase nuclei of meristematic cells of Allium cepa, Vicia faba, and Raphanus sativus are characterized by the presence of spherical bodies approximately 1 μ in diameter. These structures are Feulgen-negative but stain metachromatically with azure B, as the nucleolus, following fixation with glutaraldehyde. At the ultrastructural level, they consist predominantly of fibrils estimated to be between 70 and 100 A in diameter which greatly resemble those found within certain zones of the nucleolus in these plant species. Moreover, in Allium cepa, these spherules often exhibit dense particles which are also found within the fibrillar zones of the nucleolus in this species. The observations that the bodies in question are frequently located at the surface of the nucleolus and moreover show cytochemical and ultrastructural similarities with this organelle suggest that they may originate from the nucleolus. However, the common association of the spherules with chromosome strands may indicate instead that these bodies represent extranucleolar ribonucleoprotein materials synthesized by specific chromosomal loci.

INTRODUCTION
In the course of an earlier electron microscope study (1) of nuclear structures in meristematic cells of Allium cepa and Vicia faba, spherical bodies, approximately 2 μ in diameter, were observed at interphase. These structures were lighter than either the chromosomes or the nucleolus under electron microscopy, but stained metachromatically with azure B, as the nucleolus, following fixation with formalin. Since then, nuclear bodies of a similar appearance have been noted in Tetrahymena pyriformis (2, 3), in kidney (4, 5), and thymus (6).

The purpose of the present paper is to describe in more detail the distribution of these bodies during interphase, preprophase, and prophase and to report certain characteristics which they share with the nucleolus.

MATERIAL AND METHODS
Roots of Allium cepa grown in distilled water and of Vicia faba and Raphanus sativus L. grown in damp vermiculite were used for the present study. Portions 0.5 to 1 mm in length were excised from the root tips and fixed in the following solutions: (a) 1 per cent osmium tetroxide in distilled water (7), (b) 1 per cent osmium tetroxide buffered with Veronal (8) or phosphate (9) to a pH of 7.3, (c) 4 per cent formaldehyde or glutaraldehyde. Other roots were fixed in one of these aldehydes and postfixed in 1 per cent osmium tetroxide buffered with Veronal (8) or phosphate (9) to a pH of 7.3, (c) 4 per cent formaldehyde or glutaraldehyde. Other roots were fixed in one of these aldehydes and postfixed in 1 per cent osmium tetroxide (10). Following dehydration in alcohol the specimens were embedded in Epon 812.

Sections 0.25 to 0.75 μ thick were stained with Feulgen and methylene blue (11); ultrathin sections were stained with solutions of heavy metals (12, 13). Electron micrographs were obtained with a Siemens
RESULTS

Morphology

The small bodies described in this report are difficult to recognize under light microscopy except in rather thin preparations. The examination under phase-contrast microscopy of serial sections 0.25 μ thick shows that these nuclear structures correspond to small spheres about 1 μ in diameter.

The ultrastructure of the spherules varies with the fixation used and, to some extent, also with the species. After fixation with buffered osmium tetroxide they show a homogeneous texture in *Vicia faba* and *Raphanus sativus* (Figs. 18 to 20) and consist of tightly packed fibrils the diameter of which is estimated to be between 70 and 100 Å (Fig. 19). It is not clear how these fibrils are organized within the bodies or whether they represent their only structural constituent. At high enough magnification (Fig. 19) the boundaries of the spherules are not too clearly delineated due to the intermingling of their constituent fibrils with those of the surrounding nucleoplasm. In *Allium cepa* the bodies in question are often further characterized by the presence of dense granules (Figs. 13 and 14) of the type already described in nucleoli (1, 14-17) and prenucleolar bodies (1) of this species as well as of other plants. Many cells in *Allium cepa* contain spherical bodies which are slightly larger and may reach 1.3 μ in diameter. The rather loose organization of their fibrils (Figs. 15 and 16) confers upon these bodies a configuration somewhat reminiscent of a ball of threads (Fig. 13) and a density sometimes only slightly higher than that of the surrounding nucleoplasm.

A quite similar ultrastructural organization of

---

**Figure 1** *Allium cepa* interphase nucleus showing two peripheral heterochromatin masses (ht), one closely associated with the nucleolar surface. One light circular zone (lz) is located next to the nuclear envelope. Root tip fixed in buffered osmium tetroxide. Section 0.7 μ thick, stained by means of the Feulgen reaction and counterstained with methylene blue. X 4000.

**Figure 2** *Allium cepa* interphase nucleus characterized by the presence of a spherule (sp) adjacent to a heterochromatin mass (ht). A light circular zone (lz) is seen at the nuclear boundaries. Root tip fixed in buffered osmium tetroxide. Phase-contrast micrograph of a section 0.5 μ thick, stained by means of the Feulgen reaction and counterstained with methylene blue. X 4000.

**Figure 3** Nucleus similar to that illustrated in Fig. 2. In this micrograph the spherule is completely surrounded by heterochromatin. Buffered osmium tetroxide fixation. The section 0.5 μ thick, stained by means of both the Feulgen reaction and methylene blue, was photographed under phase-contrast microscopy. X 4000.

**Figure 4** *Allium cepa* preprophase nucleus with three nucleoli. Two spherules (sp) may be recognized on the surface of one of these organelles. The nucleolar organizing (nlo) regions of two of the nucleoli are seen within the nucleolar mass. Buffered osmium tetroxide fixation. Preparation stained and photographed as preceding figure. X 4000.

**Figure 5** *Allium cepa* preprophase nucleus depicting a light circular zone (lz) located on the surface of one of the nucleoli. Buffered osmium tetroxide fixation. Section 0.7 μ thick, stained as preceding preparation but photographed under ordinary optics. X 4000.

**Figure 6** *Allium cepa* preprophase nucleus shows one spherule (sp) and a light zone (lz). Same type of preparation as Fig. 5. X 4000.
these nuclear spherules is observed following fixation with formaldehyde, glutaraldehyde, or aldehyde-osmium tetroxide. However, their boundaries appear more continuous with the surrounding nucleoplasm when a double fixation is used, presumably as a result of a better preservation of the nucleoplasm itself.

Unbuffered solutions of osmium tetroxide in distilled water (7) give rise to marked changes in the density of the chromosomes and nucleoplasm as compared with that of the nucleolus and spherical bodies (18). The spherules are somewhat more compactly organized and their fibrillar texture less evident (Fig. 17) than when standard fixatives (8, 9) are used.

**Staining Characteristics**

The nuclear spherules are Feulgen-negative but may be brought out with methylene blue staining (Figs. 2 to 4, 6 to 10). Their density, following such staining, usually matches quite closely that of the nucleolus (Figs. 6, 7, 9, and 10). However the density of these two types of structure relative to that of the chromosomes varies somewhat with the species examined and the method of fixation used.

Following fixation with buffered solutions of osmium tetroxide, the spherules and nucleoli are lighter than the chromosomes in *Vicia faba* preparations stained with the Feulgen procedure and methylene blue. The same situation also usually prevails in *Allium cepa* meristematic cells (Figs. 2 to 4, 6, and 7) but in this species both the spherules and nucleoli may sometimes stain more densely than the chromosomes (Fig. 8); the reasons underlying their occasional increased stainability in certain preparations are not understood. Instances have also been recorded in *Allium cepa* where the spherules are lighter than both the nucleolus and the chromosomes. This last observation is in accord with the finding that some of these bodies show a rather loose ultrastructural organization (Figs. 15 and 16).

In *Vicia faba* material fixed with unbuffered solutions of osmium tetroxide in distilled water, the chromosomes stain very little with the Feulgen reaction and likewise appear to react to only a small extent with methylene blue. As a result the chromosomes are barely distinguishable even under phase-contrast microscopy. The nucleolus and the spherical bodies, nevertheless, stain quite intensely with methylene blue and are therefore easily seen in thin sections (0.5 μ). In fact, due to their relative density as compared with that of the

**Figures**

**Figure 7** Micrograph of preparation similar to Fig. 6. Here a spherule (sp) and light zone (lz) are both located next to the nuclear boundaries. × 4000.

**Figure 8** *Allium cepa* preprophase nucleus characterized by two spherules (sp) much denser than usually found in this species. Phase-contrast micrograph of section 0.5 μ thick. Same fixation and staining procedures as previous figures. × 4000.

**Figure 9** Midprophase nucleus from *Vicia faba* root tip fixed with 1 per cent unbuffered solution of osmium tetroxide containing 0.005 per cent CaCl₂. Note that the chromosomes are not too conspicuous but that both the nucleolus (nl) and the spherule (sp) are quite dense. Phase-contrast micrograph of a section 0.7 μ thick, stained by means of both the Feulgen reaction and methylene blue. × 4200.

**Figure 10** *Allium cepa* midprophase nucleus depicting two spherules (sp). Buffered osmium tetroxide fixation and staining as above figures. Phase-contrast micrograph of a section 0.7 μ thick. × 4000.

**Figure 11** Midprophase nucleus (*Allium cepa*) with a light circular zone (lz). Fixation and staining as in Fig. 10. The section 0.7 μ thick was photographed under ordinary optics. × 4000.

**Figure 12** Late prophase nucleus (*Vicia faba*) showing a dense spherule (sp) at a time when the nucleolus (nl) is dispersing. Phase-contrast micrograph of a section 0.5 μ thick. Same fixation and staining as Figure 9. × 3500.

4 *The Journal of Cell Biology* · Volume 26, 1965
FIGURE 13 Electron micrograph of portion of a preprophase nucleus (*Allium cepa*). The nucleolus (nl) is clearly seen to consist of a peripheral granular zone (gz) and a fibrillar zone (fz) located more centrally. This latter zone contains most of the dense particles seen in the nucleolus. The lighter central area shows chromatin material corresponding to the nucleolar organizer (nlo). Next to the nucleolus is recognized a spherule consisting predominantly of fibrils but also exhibiting dense particles similar to those found within the nucleolus. The chromosomes are made up of fibrils mostly and contain no such particle. Fixation with buffered osmium tetroxide. The preparation was double-stained with solutions of uranyl acetate and lead hydroxide. × 41,000.
FIGURE 14 Electron micrograph similar to Fig. 13. The majority of the dense particles are restricted to the central fibrillar zone (fz). The spherule (sp), located within the nucleoplasm, shows a number of identical particles. Note that these particles are not found free in the nucleoplasm. Other less dense particles are, however, detected in groups (arrows) between the chromosomes. Fixation and heavy metal staining as in Fig. 13. × 38,000.
chromosomes, these bodies are more easily detected in such preparations than in material fixed with buffered osmium tetroxide. When calcium ions are added in increasing concentrations to the fixing solution, the chromosomes progressively gain density (18) and become more stainable with both the Feulgen procedure and methylene blue (Fig. 9).

Under electron microscopy it is observed that the staining behaviour of the small spherical bodies likewise closely corresponds to that of the nucleolus. Following fixation with buffered osmium tetroxide and after staining with a 1 per cent aqueous solution of uranyl acetate for 2 hours, the density of the spherules equals that of the nucleolus but both types of structures are lighter than the chromosomes. Subsequent to counterstaining with lead hydroxide, an increase in the density of the two first structures is observed and it then matches that of the chromosomes (Figs. 13, 14, and 18).

Occurrence and Distribution in Interphase, Preprophase and Prophase Nuclei

In a given section 0.5 μ thick or under electron microscopy, the majority of nuclei characterized by the presence of spherules show only one such body (Figs. 2, 3, 6, 7, 9, 12 to 14, 18, and 20) but, occasionally, some nuclei exhibit two such bodies (Figs. 4, 8, and 10). A survey of consecutive sections reveals, moreover, that a proportion of nuclei, much higher than suggested by the examination of single preparations, possess at least one spherule. Taking into account the portion of a nucleus represented in a section 0.5 μ thick as well as the frequency of occurrence of these structures per section, it is estimated that approximately half of the nuclei contain one or two spherical bodies. A similar conclusion is arrived at when series of consecutive sections are examined. Indications have also been obtained that certain nuclei are characterized by three and sometimes four similar bodies.

No attempt was made in the course of the present work to investigate the possibility of variations in the frequency of occurrence of these bodies, from interphase to late prophase, in a given tissue of the root tip or of the influence of cell differentiation on this frequency.

During interphase, the nuclear spherical bodies are quite often closely associated with heterochromatin masses (Figs. 2 and 3) which sometimes surround them completely (Fig. 3). Such associations are observed both when the spherules are found on the surface of the nucleolus and when they are located elsewhere within the nucleoplasm. By preprophase, presumably due to the disappearance of these chromatin masses themselves (11), the spherules are no longer associated with heterochromatin. Spherical nuclear bodies are also found throughout prophase (Figs. 9, 10, and 18), and some are still detected at the end of that stage when the nucleolus has already begun to disperse (Fig. 12). Their size, staining characteristics, and ultrastructure (Figs. 18 and 19) do not appear to differ from the situation observed at interphase or preprophase. No such spherules are detected at metaphase, anaphase, or telophase in Vicia faba. Prenucleolar bodies similar to those
already described (1) have been observed at early and midtelophase in *Allium cepa*; they are of various sizes and shapes and, moreover, show a fibrillo-granular ultrastructure. Such bodies are thus, to all appearances, not closely related to the spherules under discussion, which are essentially fibrillar in texture (Figs. 19 and 20).

There appears to be a definite pattern in the distribution of the spherules: they are sometimes either found at the surface of the nucleolus (Figs. 4, 13, and 20) or in the immediate proximity of the nuclear envelope (Figs. 7, 9, and 16), but are more commonly observed in the nucleoplasm at intermediate positions between these two sites (Figs. 2, 3, 6, 8, 10, 12, 14, 15, 17, and 18).

Under light microscopy, interphase, preprophase, and prophase nuclei quite frequently show circular, unstained areas only slightly larger than the bodies themselves. These light zones are characterized by a distribution matching that of the spherical bodies: they are frequently observed at the surface of the nucleolus (Fig. 5), somewhere in the middle of the nucleus (Figs. 6 and 11), or at its boundaries (Figs. 1, 2, and 7). Such circular zones most likely correspond to the very loosely organized light bodies observed under electron microscopy (Fig. 16), and it is conceivable that they are in the process of disintegrating. However, it must also be noted that such bodies may remain compact in structure even though located next to the nuclear envelope (Figs. 7 and 9). Also, spherules similarly located within a given nucleus may show quite different densities (Figs. 6 and 7).

**DISCUSSION**

*Possible Relation of the Spherical Bodies with the Nucleolus*

Our observations show that the nuclear spherules share a number of morphological and staining characteristics with the nucleolus. Both types of structure react identically with methylene blue, azure B, or to solutions of heavy metals. Subsequent to such staining procedures, the spherules show a density which matches that of the nucleolus; however, the loosely organized bodies sometimes found in *Allium cepa* (Figs. 15 and 16) remain lighter. This similarity in staining behaviour is especially striking when sections are treated for increasing lengths of time with solutions of uranyl acetate. The densities of both the spherules and nucleoli are then found to be equal (Figs. 13, 14, and 18) and to increase at a similar rate.

It is observed, moreover, that the spherical bodies consist predominantly of tightly packed fibrils the diameter of which is between 70 and 100 A (Fig. 19). Such fibrillar material is indistinguishable from that observed within the non-granular zones of the nucleolus (11) (Figs. 13, 14, and 20). The ultrastructural similarity between spherules and nucleoli is further illustrated in *Allium cepa* where the former bodies and the fibrillar zones of nucleoli are characterized by the presence of dense granules some 150 to 400 A in diameter (Figs. 13 and 14). These particles have thus far been noted only within the fibrillar zones of the nucleolus (14) and the telophase pre-nucleolar bodies (1).
Finally, it should be recalled that following fixation with unbuffered solutions of osmium tetroxide the chromosomes are barely visible under light and electron microscopes, but that both the spherules and the nucleoli remain quite conspicuous (Figs. 9 and 17). A similar situation prevails in *Raphanus sativus* material fixed with buffered solutions of aldehydes, osmium tetroxide (Fig. 20), or both.

The above series of observations taken as a whole, together with the frequent presence of spherules on the nucleolar surface, (Figs. 4, 13, and 20) suggest the existence of some sort of relationship between the bodies under discussion and the nucleolus. Furthermore, on the basis of the ultrastructural characteristics of the nuclear spherules, this relationship may be assumed to exist most particularly with the fibrillar zones of the nucleolus.

Judging from the binding of uranyl ions to RNA and proteins (19) and from the progressive increase in the density of the spherules as a result of staining with aqueous solutions of uranyl acetate, it appears likely that the spherules consist mainly of RNA and proteins. This, at least, is concordant with available cytochemical data concerning the nature of the different nucleolar zones (19-24) as well as that of very similar bodies observed in *Tetrahymena pyriformis* (2, 3). The presence of RNA within the spherules is further indicated by their metachromatic staining with azure B in preparations fixed with glutaraldehyde and embedded in glycol methacrylate.

**Nature and Fate of the Spherical Bodies**

With respect to the problem of the nature of the spherical bodies, a number of possibilities must be envisaged. A first hypothesis is that the bodies in question represent remnants of the prenucleolar substance observed at telophase in *Vicia faba* (11) and of the prenucleolar bodies characterizing corresponding nuclei in *Allium cepa* (1). Since, however, this material shows a fibrillgranular texture in both species, it would have to undergo some transformation at early interphase to account for the predominantly fibrillar ultrastructure of the spherules at that stage as well as during prophase (Figs. 13, 14, 18, and 19). The available evidence, in *Vicia faba* (11) at least, suggests on the contrary that the prenucleolar material retains its fibrillgranular texture till late telophase and is completely incorporated into the maturing nucleolus. No spherical bodies are ever seen while this condensation process is taking place.

One could also conjecture that the spherules are analogous to the parachromatin material identified cytochemically within certain types of animal cells. It is believed that these nucleolar parachromatin bodies, or nucleolini, are extruded into the nucleoplasm (25, 26) and from there to the cytoplasm (26, 28). A similar migration phenomenon may account for the observed distribution of the spherules in nuclei of *Vicia faba* and *Allium cepa*, but a number of facts remain which indicate that the parachromatin bodies do not correspond to the bodies under discussion. First, as far as we know, nucleolini have not been demonstrated cytochemically in plant meristematic cells and, moreover, no corresponding structures are recognized under the electron microscope (1, 11, 14, 15) within the nucleolus. Secondly, the parachromatin bodies, contrary to the spherules, are thought to increase in size within the nucleoplasm during prophase and to persist in the cytoplasm (27, 28) throughout metaphase, anaphase, and sometimes telophase. Till these inclusions are characterized at the ultrastructural level, it will be difficult to ascertain their relationship to the various components of the nucleus.

Further studies will also be required to verify whether the spherules described here are related to the micronucleoli seen in plant cells by certain authors (29, 30).

In keeping with their frequent localisation on the surface or in the immediate proximity of the nucleolus during interphase and prophase, it is conceivable that the spherules originate from this organelle. This hypothesis derives support from
the observed similarities in ultrastructure between the spherules and the fibrillar zones of the nucleolus (Figs. 14 and 20) as well as from reports that vacuoles and amorphous substances (31–34) are extruded from the nucleolus in animal and plant cells. Our own data do not demonstrate unequivocally that the spherules seen in Allium cepa, Vicia faba, and Raphanus sativus actually originate from the nucleolar mass. These observations are equally compatible with the formation of such bodies directly on the surface of the nucleolus as a result of the activity either of the nucleolus itself or of the nucleolus-associated chromatin.

A final assumption which must be considered is that these nuclear bodies represent extranucleolar material synthesized by specific chromosomal loci. In view of the apparent production of these bodies throughout interphase (Figs. 1 to 8, 13 to 16) and prophase (Figs. 9 to 12, 18), it must also be conjectured, if this hypothesis is correct, that we are dealing with loci which become synthetically active subsequent to the formation of the nucleolus at telophase. The striking ultrastructural resemblance of these bodies with the fibrillar zones of the nucleolus could thus reflect that these two types of nuclear bodies represent different forms of chromosomal products which consist predominantly of similar biochemical constituents. The
FIGURE 20 Portion of *Raphanus sativus* nucleus illustrating a spherical body (sp) located on the surface of the nucleolus (nl). At this magnification, the texture of the spherule is seen to resemble that of the fibrillar zones (fz) of the nucleolus. In this species the nucleolus also consists of granular zones (gz) intermingled with fibrillar areas.

The nucleoplasm appears homogeneous and the chromosomes are indistinguishable. X 48,000.
available cytological data pertaining to nucleoli (19-24), as well as our preliminary observations with azure B staining, point to RNA as being one of the constituents which they contain in common. It may well be, therefore, that the spherules described in the present report are analogous in certain respects to the ribonucleoprotein materials elaborated by lambrush (35) and salivary gland (36, 37) chromosomes. Further studies have been initiated with cytological and autoradiographic techniques in combination with electron microscopy to characterize further the chemical nature of the spherules and to verify their possible relationship to the nucleolar constituents.

Now, concerning the fate of the spherules, it appears likely that they are eventually secreted into the cytoplasm. They are indeed frequently observed at the periphery of the nucleus, and many of our micrographs are best interpreted by assuming that they disperse in this area (Figs. 1, 2, 7, and 16). The fact that in the present study such bodies have never been seen in the process of migrating across the nuclear envelope most likely indicates that transport of material takes places at the macromolecular level contrary to the situation observed in Tetrahymena pyriformis (2, 3) where similar structures are extruded by means of blebs of this envelope.

This investigation was supported by grant T-1018 of the National Research Council of Canada.

Received for publication, April 14, 1964.

BIBLIOGRAPHY


