Structure and Mode of Formation of the Nucleolus in Meristematic Cells of *Vicia faba* and *Allium cepa*

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ABSTRACT

Interphase nucleoli from *Vicia faba* and *Allium cepa* meristematic cells are roughly classified into two categories: (*a*) those that commonly show a rather homogeneous texture (except for small light spaces of various sizes) and frequently contain dense particles 140 A in diameter; (*b*) those found more frequently in *Vicia* characterized by a very sharp boundary between a dense outer cortex and a much lighter central core. The dense particles are not found in such nucleoli. In *Allium* the boundary is more irregular and dense particles are sometimes observed in the outer layer. Many nucleoli show a structure intermediate between these two types. They are characterized by a gradient of increasing density from the center to the periphery and occasionally contain dense 140 A granules.

During interphase, certain nucleoli are closely associated with segments of chromatin strands which undoubtedly represent nucleolar organizing regions.

The dense 140 A granules are followed during the mitotic cycle. In *Allium*, they are first seen in loose clusters between arms of late anaphase chromosomes where they become more concentrated in early telophase. The substance within which they are scattered slowly increases in density during that time until finally, the particles are limited to small bodies of distinctive character. Evidence is presented suggesting that these small prenucleolar bodies fuse during telophase to give rise to the mature interphase nucleoli.

Similar events are described in *Vicia* material except that a coating of dense substance appears around telophase chromosomes before the formation of prenucleolar bodies.

INTRODUCTION

The structure of interphase nucleoli in meristematic cells of *Vicia faba* and *Allium cepa* was described in a previous note (1). At that time, special emphasis was placed on the occurrence of dense granules, 140 A in diameter, in many interphase nucleoli after fixation with osmium tetroxide. In the onion, such particles were reported to be already present in late anaphase and early telophase. It was pointed out, also, that as far as could be observed with the electron microscope, the distribution of prenucleolar material during early telophase in *Vicia faba* was different from that in *Allium cepa* material.

In the present study the structure of nucleoli, after fixation with osmium tetroxide, is reported in more detail and morphological variations introduced by formaldehyde and Carnoy's fixative are also briefly discussed.

Material and Methods

Roots of *Allium cepa*, grown in tap water, and of *Vicia faba*, grown in Bonner's medium (2), were used for this study. Just before fixation, they were cut to a length of 2 to 3 mm. and sometimes split in two longitudinally. Most of the work was done on material fixed with buffered (pH 7.4) osmium tetroxide (3), but 5 per cent formaldehyde (buffered to a pH of 7.4 with

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veronal acetate) and Carnoy's fixative were also used on a limited number of specimens.

For electron microscopy, the material was embedded in *n*-butyl methacrylate and sectioned with a Porter-Blum microtome. The sections were mounted on carbon-coated grids and examined with an RCA-2A electron microscope.

Thick sections (2 μ) were also prepared and stained with azure B or by the Feulgen procedure. Staining with azure B (buffered to a pH of 7.4 with veronal acetate) was carried out for a period of 3 hours and was followed by differentiation overnight in tertiary butyl alcohol. At this pH, azure B is not necessarily specific for nucleic acids. For Feulgen staining, sections fixed with osmium tetroxide were hydrolyzed 20 minutes, those fixed with formaldehyde and Carnoy's fixative, 12 minutes (4).

RESULTS

The structure of the nucleolus will be described first in interphase nuclei from *Vicia* and *Allium* root meristems. Then, changes taking place during other stages of mitosis will be presented separately as they occur in these two plants.

Structure of the Interphase Nucleolus

General Morphology:

Interphase nucleoli are very conspicuous and often occupy a large part of the volume of their respective nuclei; their diameter ranges from 4 to 8 μ. They vary greatly in gross morphology, their profiles often being quite irregular but, in three dimension they usually appear as more or less ellipsoidal bodies. Their periphery is corrugated and forms a large number of small indentations. Chromosomal material is usually found projecting into these small inpocketings and there often is actual contact between the chromosomes and the nucleolus at these places, as well as at the extremities of some of the indentations (Figs. 1 and 5). There may sometimes be two separate nucleoli per nucleus and, occasionally, they appear to have fused to give rise to a dumbbell type of structure.

It is possible to distinguish a number of different materials in interphase nucleoli and, as will be shown shortly, the latter may be classified into types according to the presence and distribution of these substances.

(*a*) The main constituent of interphase nucleoli matches the chromatin strands in density and, at low magnification, appears almost homogeneous (Fig. 1). However, examination of images at higher magnification reveals that this nucleolar substance consists of what appear to be closely packed granules each 100 to 150 A in diameter.[1] Favorable pictures suggest that these apparent granules are profiles of tubules or possibly of paired fibrils of smaller diameter.

(*b*) The second nucleolar constituent can be characterized in more precise terms; it is a quite dense, doughnut-shaped particulate component (1) whose diameter ranges from 70 to 200 A. The majority of such particles are approximately 140 A in diameter.

(*c*) Finally, a third component may be distinguished in certain interphase nucleoli. It is much lighter than the other two constituents and appears to consist of dispersed granules whose diameter is 100 to 150 A. The latter resemble the granules forming substance (*a*), above, and it is possible that both constituents (*a*) and (*c*) differ only in the degree of packing of these granules. The matrix within which these structural elements are scattered is not resolved in our pictures. Also, depending on the dilution of the granules themselves this third nucleolar substance may become quite structureless in certain nucleoli and in these cases it is difficult to identify as such.

Interphase nucleoli may be roughly classified into two types according to the distribution of these three components.

In *Vicia*, about two-thirds of the nucleoli are rather homogeneous and compact in structure and consist mainly of component (*a*) (Fig. 1). This is made more obvious in images at high magnification (Fig. 2) where the densely packed granules are more easily seen. These nucleoli contain many small internal light spaces and sometimes a number of much larger ones, some 0.3 μ in diameter. These spaces vary in size, shape, and number in different nucleoli but seem to represent a normal part of their internal structure (Figs. 1, 3, 4, 5, and 7). Approximately half of these homogeneously dense nucleoli contain a large number of the dense 140 A granules, *i.e.* component (*b*) (Figs. 3 and 4), whereas the others do not (Figs. 1 and 2). As far as can be observed no other difference in structure exists between these dense nucleoli with or without the 140 A granules and, likewise, no significant difference in the general appearance of their respective nuclei can be detected. Component (*c*) is

[1] Granules of similar diameter have been observed in nucleoli by previous authors (14, 35). However, these particles as well as the smaller ones also reported (36) were recently interpreted as gyres of helicoidal filaments in the nucleolus (37).

not obvious in this first type of nucleolus and if it does exist at all one must assume that it is to be found in the lighter spaces and possibly in the matrix material within which constituents (*a*) and (*b*) are embedded.

The second category of nucleoli presents an interesting organization; they consist of two quite different zones separated by a rather sharp boundary (Figs. 5 and 6). A light core is present in the center and is surrounded by a cortex of much greater density. Except for the many light spaces similar to the ones found in most nucleoli, the texture of this outer region matches that of the first group of nucleoli described above and thus consists mostly of component (*a*) (*i.e.* compare Fig. 6 with Fig. 2). In *Vicia* the boundary between these two phases is usually very sharp (Figs. 5 and 6) and quite regular in outline except for small indentations all around. Occasionally the nucleolar cortex is not homogeneous but exhibits a greater density at the inner and outer boundaries. The dense 140 A granules, as a rule, are lacking in this type of nucleolus. The central portion of such nucleoli appears to consist of component (*c*) exclusively but it is possible that the dispersed granular elements which it contains are in reality the same as those which are tightly packed in the cortex. In some nucleoli at least, the particles found in both regions look quite similar.

Many nucleoli show a structure intermediate between the two categories described above. In general these nucleoli are characterized by an increase in the concentration of component (*a*) from the center outwards (Figs. 7 and 8). Some contain 140 A particles in varying amounts (Fig. 8) whereas others do not (Fig. 7). When present at all, there seems to be a tendency for these particles to be more concentrated in the densest region of the nucleolus (Fig. 8). The density gradient present in these nucleoli may reflect the actual distribution of their material but such images could also represent grazing sections through the cortical region of nucleoli similar to those illustrated in Figs. 5 and 6. However, dense granules (140 A) have not been observed in *Vicia* nucleoli of this last type and one would therefore not expect to find any in Fig. 8.

In *Allium cepa* material, most of the nucleoli observed belong to the two types found in *Vicia*. However, a few differences exist in the case of the nucleoli which consist of two well separated phases. In the onion, the boundary between these two nucleolar regions is less regular than in *Vicia* and, moreover, dense 140 A particles are sometimes found in the cortex (Fig. 9). In view of this last morphological difference it is not clear whether we are dealing with entirely homologous nucleoli in these two plants.

A survey of nuclei in *Vicia faba* and *Allium cepa*, containing two or more nucleoli, reveals that the latter are always of the same type within any given nucleus. Also, whenever present, the dense 140 A particles are found in all nucleoli. It would thus appear that the morphological variations observed between different types of nucleoli are significant; they either reflect some sort of differentiation of the nuclei or different states of physiological activity of the nucleoli themselves.

Interphase nuclei in *Allium cepa* often show small bodies of much less density than the nucleoli regularly observed during that stage (Fig. 10). They are approximately 2 μ in diameter and, in serial sections, are found to be spherical in shape. Besides this difference in size, these bodies are characterized by a very homogeneous, almost amorphous texture and by a density only slightly superior to that of the nucleoplasm. In a given section no more than two such bodies are observed. Thick sections stained with azure B show small bodies of a similar size which stain metachromatically as do the big nucleoli.

Fixation with Formaldehyde and Carnoy's Solution.—In material fixed with formaldehyde and Carnoy's solution the morphology of the nucleus as a whole and of the nucleolus differs quite markedly from that observed after osmium tetroxide fixation. The finer texture seen in the chromatin and nucleolus after this last fixative is lost and a rather amorphous structure is observed instead.

After fixation with formaldehyde, the chromatin strands become lacunar and coarse in texture. Most nucleoli show the cortex and core type of organization but the respective densities of both these regions are reversed (Fig. 11) as compared to those observed after fixation with osmium tetroxide (Figs. 5 and 6). Their central region is now more compact that the cortex but does not quite match the chromatin in density. The light microscope presents a picture which is consistent with these observations. It is found, after staining with azure B for instance, that the staining intensity is different in distribution from that found in osmium-fixed material. Instead of a homogeneous staining or, in some cases, a decreasing gradient of staining towards the center, a ring of less densely stained material is now observed at the periphery of most

nucleoli. The 140 A granules have also disappeared and, instead, a few large (300 to 700 A) particles of corresponding density are now observed, mostly in the nucleolus (Fig. 11). The similarity in the location and density of these two types of granules suggests that they both either represent precipitated products of a specific nucleolar substance which reacts differently in osmium tetroxide and formaldehyde solutions, or that clumping of the 140 A granules takes place, in this last fixative, to give rise to larger particles.

Except for a similarly amorphous texture, the appearance of nucleoli in material fixed in Carnoy's solution is different from that just described. Examination with the light microscope of 2 μ sections prepared from methacrylate blocks shows many small refractile bodies which appear to be located slightly above the nucleoli, where they can be seen before as well as after staining. Thin sections examined with the electron microscope do not show any of the dense bodies, but instead a number of small holes are observed in the otherwise homogeneous structure of the nucleoli. These observations suggest that during fixation with Carnoy's solution some nucleolar components are clumped into hard refractile bodies which are slightly displaced by the knife while cutting 2 μ sections and which are removed completely during the preparation of thinner sections. Since the 140 A particles are absent it is possible that they are precipitated into the big refractile bodies now observed with the light microscope. It is evident that more work will have to be done in order to determine whether or not the particles of different appearance, observed after the three types of fixation mentioned, correspond to the same nucleolar substance.

Nucleolar Organizers.—Many profiles of nucleoli, in *Allium cepa*, are characterized by a large central light area partly filled with material of chromatin texture and density. Favorable sections show crescentic profiles and indicate that the material in the nucleolar cavity is continuous with a chromatin strand in the nucleus (Figs. 14 and 15). Even in the numerous cases (Fig. 13) in which the angle of sectioning does not permit the establishment of a direct relationship between the intranucleolar material and a chromatin strand, its chromatin origin can be shown on account of its lack of the dense 140 A granules which are found in the nucleolar material wrapped around it. This follows from our observation that, during interphase, these particles are always located in bodies which are easily identified as nucleoli. Figs. 13 and 14 thus represent transverse and longitudinal sections of a cup-shaped nucleolar body into the cavity of which a chromatin strand projects.

The situation is somewhat different in *Vicia faba* material. Interphase nucleoli showing such a close association with a chromatin strand were encountered much less often and the dense 140 A particles so characteristic of such nucleoli in *Allium* (Figs. 13 to 15) were not seen in any of the cases observed. For that reason it is not always possible to distinguish clearly the chromatin from the nucleolar material. Nevertheless the nucleolus is sometimes denser than the chromatin strand which projects into it and in some favorable cases the lighter material inside the nucleolus is seen to be continuous with a chromatin strand in the nucleus (Fig. 16).

In both *Vicia* and *Allium* the segment of the chromatin strand to which the nucleolus is attached most probably represents the nucleolar organizing region of an interphase chromosome.

Variations in the Structure of the Nucleolus during the Mitotic Cycle

Prophase:

Prophase nucleoli usually show extremely irregular outlines and, in many instances, chromosomal material projects deeply within their mass (Fig. 12). They contain many light spaces of various sizes, as during interphase (Fig. 4). Dense 140 A granules are occasionally seen, but in the majority of prophase nucleoli they are lacking. In addition, nucleoli characterized by the cortex and core type of structure seen during interphase were observed only on rare occasions.

Metaphase and Anaphase:

No indication of the occurrence of nucleolar material was found from late prophase to late anaphase in either *Allium cepa* or *Vicia faba*. In the onion, however, dense granules are first observed in the form of loose clusters between the arms of late anaphase chromosomes (Figs. 17 *a* and 17 *b*). At that time they are rather diffusely distributed but seem nevertheless to be limited to certain light interchromosomal regions. In early telophase, the granules are still associated into loose clusters but their number has increased (Fig. 18). The density of the regions in which they are first seen in late anaphase is similar to that of the spindle area (Figs. 17 *a* and 17 *b*), but it in-

creases gradually and in early telophase is observed to be superior to that of the rest of the interchromosomal areas (Fig. 18).

No indication of such granules was observed at this stage in *Vicia faba.*

Telophase:

In *Allium,* as the cell plate begins to form, the dense granules are seen to agglomerate further and are now contained in denser areas. Small dense bodies scattered between and sometimes within chromosomal masses soon appear and they are then seen to contain the 140 A granules exclusively (Fig. 19). At a slightly later time, when the chromosomes begin to unravel, these bodies are of larger sizes (Fig. 20); it is assumed that this increase is due to the fusion of the smaller bodies seen previously. Finally, at the end of telophase only one or two large nucleolar masses remain presumably as a result of a continuous process of coalescence.

In *Vicia faba* the prenucleolar substance first appears in a slightly different manner. In early telophase the chromosomes are much more tightly packed than in corresponding nuclei of *Allium cepa* and as a result, only narrow light spaces are observed between them at that time (Figs. 21 and 22). In such nuclei it is occasionally possible to detect a thin layer of material squeezed between the chromosomes, but it is not clear whether this substance is actually coating the surface of the chromosomes or is just tightly packed between them. However, at places one gains the impression that it is disposed in perforated sheets or perhaps in sheets of varying thickness (Figs. 21 and 22). At a slightly later time, as the chromosomes become more completely enclosed within the nuclear envelope and the nucleus assumes a more regular shape, many new bodies are found scattered within it. They vary widely in shape and size, there often being one large nucleolar body together with five, six, or more other much smaller ones that are easily distinguished from the surrounding chromatin material. As in *Allium cepa,* all these structures can definitely be identified because of the presence of a large number of the dense 140 A granules.

In the course of telophase many light small spaces develop between and within the chromosomes which lose some of their compactness and unravel at places resulting in the appearance of narrow (0.1 to 0.2 μ) strands. Concomitantly, a coalescence of the hitherto scattered prenucleolar bodies seems to take place and a few much larger nucleolar-like structures appear. As are the smaller bodies, these newly formed nucleoli are characterized by many dense 140 A particles. In more advanced telophase nuclei the small nucleolar masses have disappeared altogether, presumably to form the big nucleoli now observed. By then the nucleus has swollen further, the chromosomes have unravelled completely and given rise to narrow strands (0.1 to 0.2 μ) forming a coarse chromatin network. The cell plate is now also more or less completely formed and the daughter cells well separated.

DISCUSSION

The Structure of the Nucleolus:

Many of the nucleoli observed in the course of this work were found to consist of different substances whose distribution often gives rise to more or less distinct nucleolar phases.

Zirkle (5) suggested long ago that the nucleolus is composed of two substances of different refractive indices and recently developed cytological techniques have allowed this problem to be studied in more detail. Chayen *et al.* (6), using ultraviolet absorbtion techniques, for instance, concluded that *Vicia faba* nucleoli are formed of an inner core and an outer zone, the latter being limited by a well defined skin. Interference microscopic observations by Davies *et al.* (7) also led to similar results, with the outer region of the nucleolus being shown to be much denser than the central region.

The greater resolving power of the electron microscope has now demonstrated these two zones more clearly in certain nucleoli (Figs. 5 and 6). The greater density of the cortex as shown by interference microscopy (7) is reflected in our images by a much tighter packing of its structural elements as compared with that of the core. The apparent "skin" (8) between the two zones can be assumed to result from the regularity and sharpness of the interzonal boundary (Figs. 5 and 6).

The concept of two nucleolar phases is not as convincingly illustrated in nucleoli which show a gradient of density outwards (Figs. 7 and 8) and still less in those which appear almost homogeneously dense (Figs. 1 to 3). However, the great majority of the nucleoli observed showed a number of lighter spaces of various sizes the largest of which might possibly correspond to the vacuoles previously reported (9–13, 21). Although some of these light spaces appear structureless, if not empty, they are not necessarily so in the living

state: their content is probably extracted during the preparation procedures.

It is interesting in this respect to note that the structural elements may be distributed quite differently within the nucleolus depending on the fixative used. Bernard *et al.* (14), for instance, found that formaldehyde and Carnoy's solution give rise to a redistribution of the nucleolar material. The first fixative is reported to produce a pseudo "pars amorpha" sometimes forming a ring similar to the nucleolus-associated chromatin as seen in the electron microscope, whereas after fixation with Carnoy's solution there are two concentric zones in the nucleolus. Our observations show that formaldehyde brings about the formation of a ring of lighter density around most nucleoli. This is also reflected in a different distribution of the azure B staining intensity as compared to nucleoli fixed with osmium tetroxide.

Nature of the 120 A Particles.—Very little can be said at present concerning the nature of the dense granules found in certain nucleoli. Particles of similar density and shape have been demonstrated in nucleoli of *Amoeba proteus* (15). However, they are bigger than those seen in our material and are reported to be also present in the cytoplasm where they vary more in size. As pointed out before (1), it is possible that they do not exist as such in the living cell and are produced during the dehydration or fixation procedures. If this is the case, it is nevertheless necessary to assume that a definite chemical component of the nucleolus clumps into granules under the influence of these external agents. It could also be that this same substance produces the bigger granules observed after both formaldehyde (Fig. 11) and Carnoy's fixative. Densitometric measurements would probably indicate whether these particles contain high atomic weight elements or metals which have sometimes been detected in nucleoli (16, 17).

Mode of Formation of the Nucleolus:

The problem of the origin of the nucleolus and its relation to the chromosomes has been studied by a number of workers in the past. Van Camp (18), first described the fusion of small nucleolar-like globules in telophase to give rise to a bigger nucleolus and Heitz (19, 20), working with *Vicia faba*, later on discovered that the nucleolus arises in telophase in close association with the secondary constriction on a particular pair of chromosomes, the satellite chromosomes. Shortly thereafter, McClintock (21) introduced the concept that organizer regions on these "nucleolar chromosomes" are directly involved with the formation and condensation process of the nucleolus.

Nucleolar material has sometimes been thought to originate from the matrix substance of the chromosomes (21, 22–24). This idea resulted mainly from the belief that the matrix forms and disappears at the same time that the nucleolus dissolves at prophase and condenses at telophase. This same material has also been reported to persist during metaphase and anaphase in close association with the chromosomes (25–28). Estable and Sotelo (26), for instance, have described filamentous structures in interphase nucleoli which were followed throughout the different stages of the mitotic cycle.[2] They suggest that these nucleolonemata unfold during prophase, stretch along each anaphase chromosome, and come together again to form the interphase nucleolus. Rattenbury and Serra (28) put forward a similar hypothesis; they believe that the nucleolus originates from a prenucleolar substance, first appearing either in the form of a coating over the chromosomes (*Vicia faba*), or as numerous spherical droplets as in *Allium cepa*, in which layers over chromosomes are also observed. These masses of prenucleolar material are irregular in shape and fill some of the lacunae between telophase chromosomes. Although their results are based on the use of stains whose specificity for prenucleolar material is not well understood, the observations of these two groups of workers are surprisingly similar to those reported here which were made with the electron microscope. As with these authors, a layer type of prenucleolar material was found in early telophase nuclei of *Vicia faba*, but so far has not been seen in late anaphase in *Vicia* and never in *Allium* as they reported. Liekwise the spherical droplets which they observed in *Allium* could very well correspond to the small bodies seen in our material during telophase. At the present time we have unfortunately no direct evidence that the thin coating observed in early telophase (*Vicia*) is related to the nucleolonemata which Estable and Sotelo (26) have seen stretched along anaphase chromosomes or to the coating described by Rattenbury and Serra (28). However, this coating is observed at the stage of mitosis and at the location where these two groups of workers saw a

[2] These filamentous structures first observed by Estable (29) have since been studied by a number of workers both with the light microscope (30–32) and the electron microscope (33–37).

similar structure and, moreover, slightly more advanced nuclei were shown in this study to be already characterized by small prenucleolar bodies. It could thus be assumed that the coating is transformed directly into these prenucleolar bodies during telophase and that, concomitantly, the dense particles also make their appearance.

The evidence is somewhat clearer in the case of the small bodies themselves. The assumption that they are prenucleolar in nature is based on the following observations. First, many telophase nuclei contain a big nucleolus as well as many small prenucleolar bodies and it is then easily ascertained that they all have a similar texture and density and also represent the only nuclear regions in which the dense 140 A particles are to be found. During interphase these particles were shown to be located in the nucleolus only. There seems therefore to be little doubt that they also characterize nucleolar material during telophase. Consequently, these particle-containing bodies can be considered truly prenucleolar in nature. This conclusion is further supported by the observation that, during maturation of telophase nuclei (as determined by the unraveling of the chromosomes and degree of reconstruction of the cell plate), the number of such bodies decreases while they enlarge correspondingly in size (Fig. 20). The mature interphase nucleolus seems therefore to derive from the smaller bodies appearing in early telophase.

In spite of the fact that the dense 140 A granules could be traced back, in *Allium*, to late anaphase at which time they are loosely scattered between chromosome arms, no definite information was obtained on the nature of the background material in these same areas. The only thing that can be said is that the interchromosomal material, within which the dense granules are located, increases in density progressively from late anaphase to middle telophase. It becomes very similar to the matrix of the prenucleolar bodies and it is not unlikely that the latter is indeed derived from this interchromosomal substance.

Nucleolar Organizer.—Since the classical work of Heitz (19) and McClintock (21) on the role of certain segments of chromosomes in the formation of nucleoli during telophase and interphase, many reports have appeared describing small chromatin inclusions (6, 38–43) and Feulgen-positive nucleolonemata (44) within certain nucleoli and evidence has also been presented for the existence of chromatin threads attached to and sometimes traversing nucleoli (6, 38–40, 45). These different observations bear out the fact that chromatin filaments are often intimately associated with the nucleolus. Electron microscopic observation has now added more weight to these findings by demonstrating clearly that during interphase segments of chromatin strands indeed penetrate within certain nucleoli and are closely associated with them (Figs. 13 to 16). This finding suggests that the small prenucleolar bodies coalesce onto special loci of these chromatin strands to give rise to the mature interphase nucleoli. It is interesting to note that the cup-shaped nucleoli observed were usually smaller than the big interphase nucleoli and therefore they could represent a certain stage of their growth.

The problem of the relationship of the prenucleolar bodies to the telophase chromosomes as well as their mode of coalescence could be more easily studied by means of serial sections and by using consecutive thick and thin sections in conjunction with specific cytochemical tests for the prenucleolar material. In the absence of this information, our results cannot yet be correlated with earlier findings (19, 20) that the prenucleolar substance condenses on certain well defined sites of the telophase chromosomes.

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Explanation of Plates

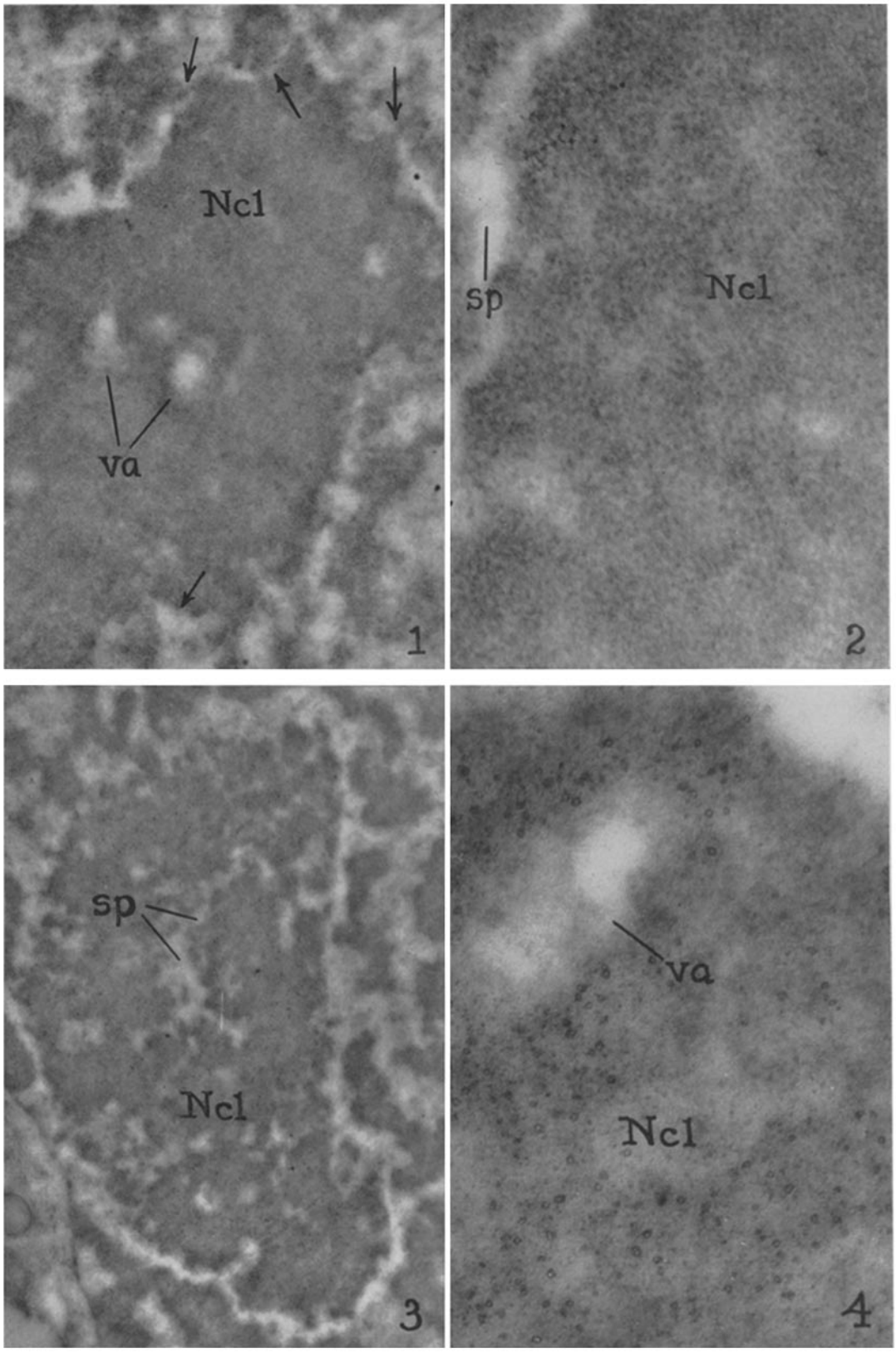
Plate 392

Fig. 1. An interphase nucleolus (*Ncl*) of the first type (*Vicia faba*) showing a rather homogeneous texture throughout except for a few vacuole-like spaces (*va*) of much lesser density. Note how closely the chromatin material follows the contours of the nucleolus at the upper left-hand corner and top of the picture. At many places there appears to be actual contact of the chromatin masses with the nucleolus (arrows). Osmium tetroxide fixation. × 19,500.

Fig. 2. A picture at higher magnification (*Vicia faba*) illustrating the closely packed texture which characterizes many nucleoli. The finest units observed appear either as small tubules approximately 150 A in diameter or paired elements of the same total diameter. A narrow light space (*sp*) separates the nucleolus from the chromatin. Osmium tetroxide fixation. × 80,000.

Fig. 3. In this micrograph light spaces (*sp*) seem to subdivide the nucleolus (*Ncl*) into irregular fragments. Dense granules are scattered throughout the nucleolus but are not present in the lighter areas. Osmium tetroxide fixation. × 13,000.

Fig. 4. A picture at higher magnification showing vacuoles (*va*) within the nucleolus (*Ncl*) in *Vicia faba*. The boundary of these light areas is quite irregular and there appears to be little if any material left inside. This nucleolus is also characterized by a large number of dense granules and it is interesting to note their distribution in the neighborhood of the vacuole. Osmium tetroxide fixation. × 85,000.



(Lafontaine: Nucleolus in meristematic cells)

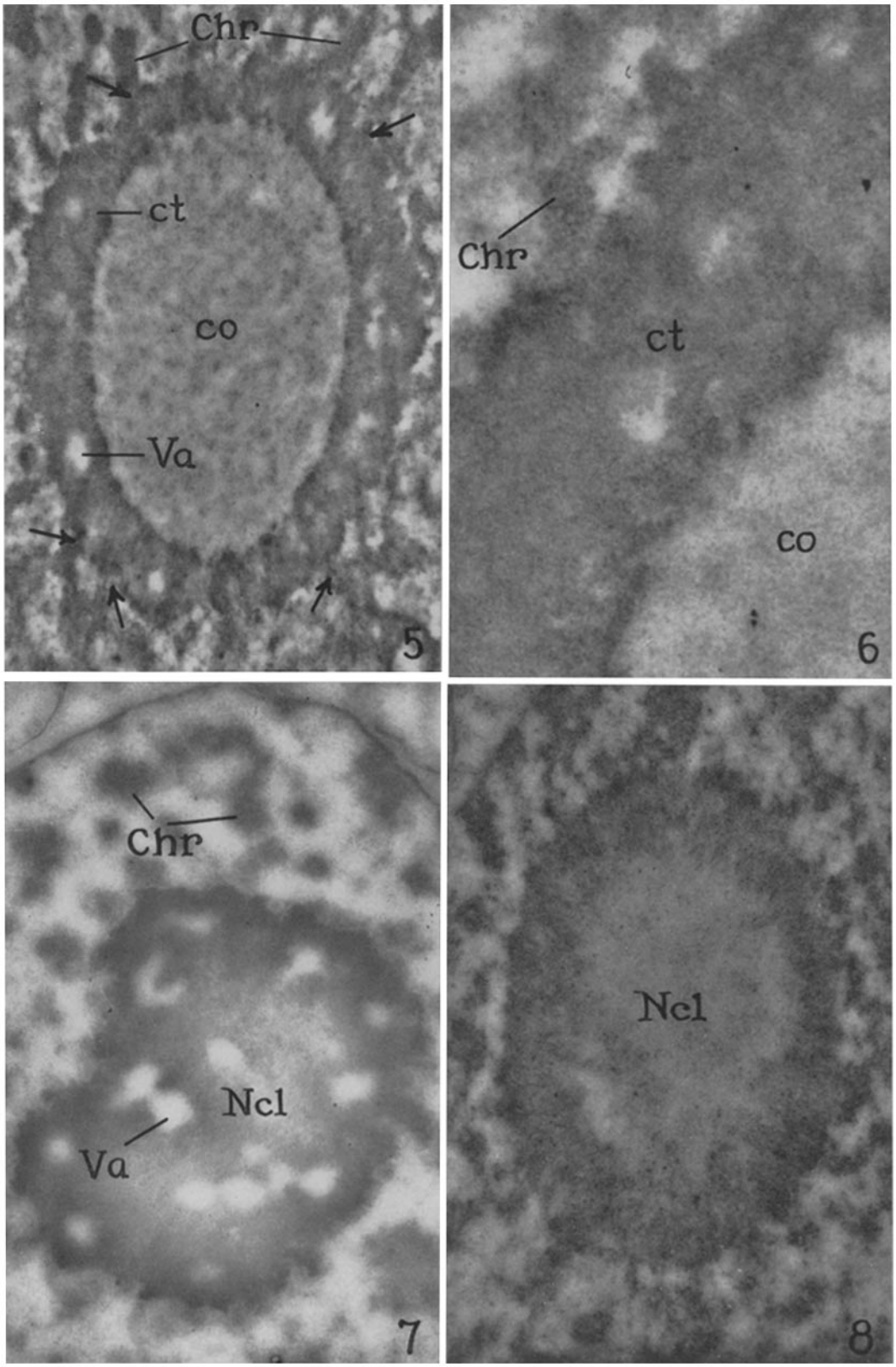
PLATE 393

FIG. 5. An interphase nucleolus of the second type in *Vicia faba*. It consists of a cortex (*ct*) of dense material disposed around a light core (*co*). The density of the cortex, except for small vacuoles (*Va*), corresponds very closely to that of the chromatin strands (*Chr*). The inner core is of a quite uniform density, slightly greater than that of the interchromatin material in the nucleus. Many of the strands are closely associated with the periphery of the nucleolus (arrows). Osmium tetroxide fixation. × 15,000.

FIG. 6. A picture at higher magnification illustrating the sharpness and regularity of the inner boundary in this type of nucleolus (*Vicia faba*). The finer structure of the cortex (*ct*) is identical to that observed in nucleoli of the first type (see Fig. 2). The texture of the core (*co*) is quite uniform and regular. A chromatin strand (*Chr*) is seen closely associated with the outer boundary of the nucleolus. Osmium tetroxide fixation. × 44,000.

FIG. 7. A nucleolus (*Ncl*) (*Vicia faba*) showing a gradient of density from the center outwards. The outermost portion of the nucleolus matches the chromatin strands (*Chr*) in density. The vacuoles (*Va*), as in other types of nucleoli, appear quite empty and of lesser density than the interchromatin material in the nucleus. Osmium tetroxide fixation. × 15,700.

FIG. 8. A *Vicia faba* nucleolus similar to the one illustrated in Fig. 7 except for the presence of many dense granules. Note the distribution of the particles and their larger concentration in the denser outermost region of the nucleolus. Osmium tetroxide fixation. × 23,000.



(Lafontaine: Nucleolus in meristematic cells)

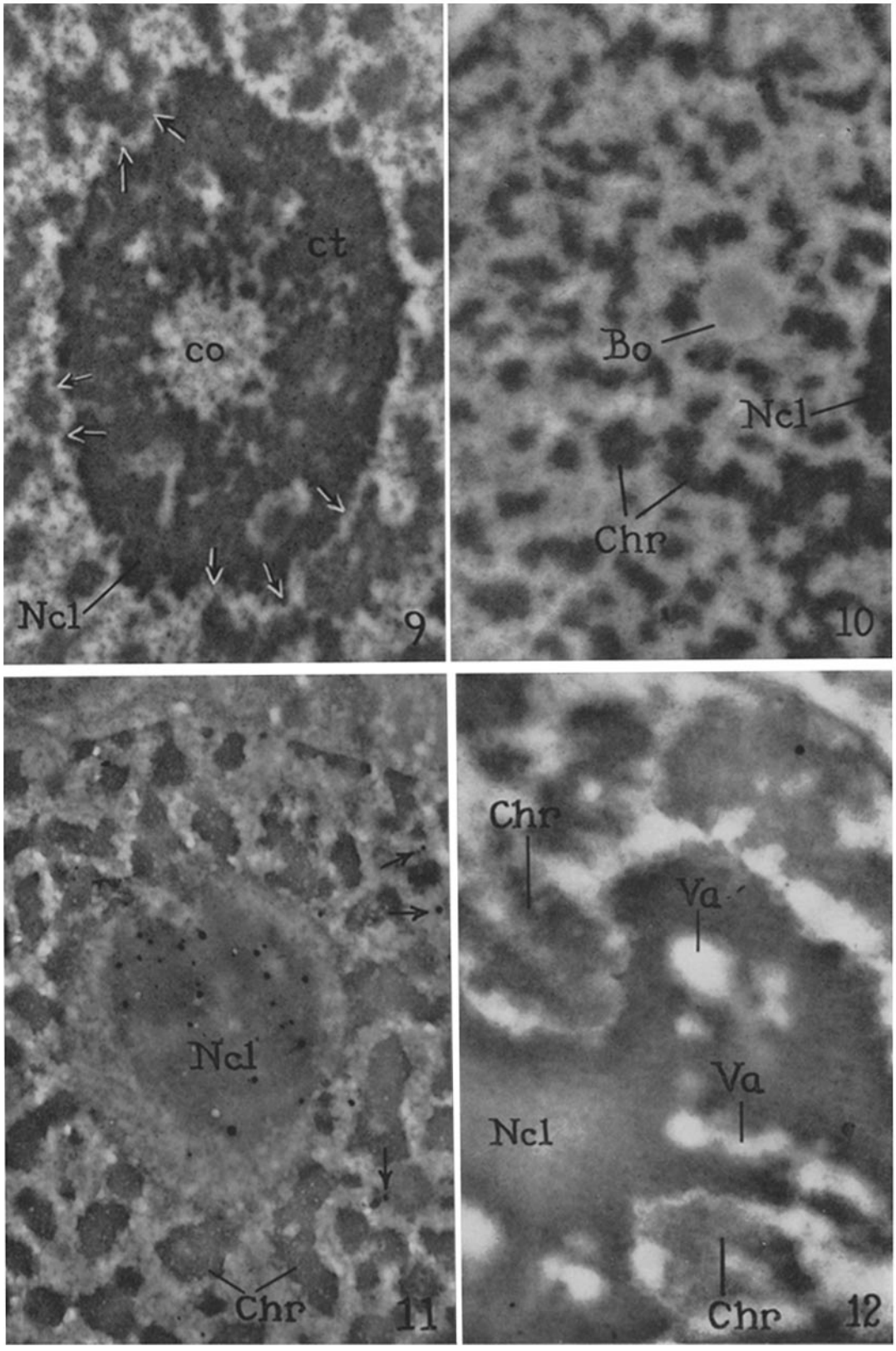
PLATE 394

FIG. 9. An interphase nucleolus (*Ncl*) of the second type in *Allium cepa*. Here the boundary between the cortex (*ct*) and the core (*co*) is much less regular than in *Vicia*. Also, dense granules are sometimes found in the cortex of these nucleoli in *Allium*. Note how closely the chromatin material follows the contours of the nucleolus at certain places (arrows). Osmium tetroxide fixation. × 22,000.

FIG. 10. An interphase nucleus showing one of the light bodies (*Bo*) which are sometimes observed in *Allium cepa*. Its density is only slightly superior to that of the interchromatin material and much lower than that of either the chromatin material (*Chr*) or of the nucleolus (*Ncl*). Osmium tetroxide fixation. × 17,000.

FIG. 11. *Allium cepa* nucleus fixed in 5 per cent buffered formalin. Here the nucleolus (*Ncl*) consists of two zones of different densities. The central part is now almost as dense as the chromatin strands (*Chr*) and the outer portion only slightly superior to that of the interchromatin material. Most of the dense particles (300 to 700 A) are found in the central portion of the nucleolus but a few of them are also scattered outside the nucleolus (arrows). × 17,0000.

FIG. 12. A prophase nucleolus (*Ncl*) in *Vicia faba* showing two deep indentations into which parts of chromosomes (*Chr*) penetrate. Note the large vacuoles (*Va*) inside the nucleolus. Osmium tetroxide fixation. × 19,000



(Lafontaine: Nucleolus in meristematic cells)

PLATE 395

FIG. 13. An electron micrograph showing the intimate relationship existing between certain nucleoli (*Ncl*) in *Allium* and one of the chromatin strands (*Chr*) of the interphase nucleus.

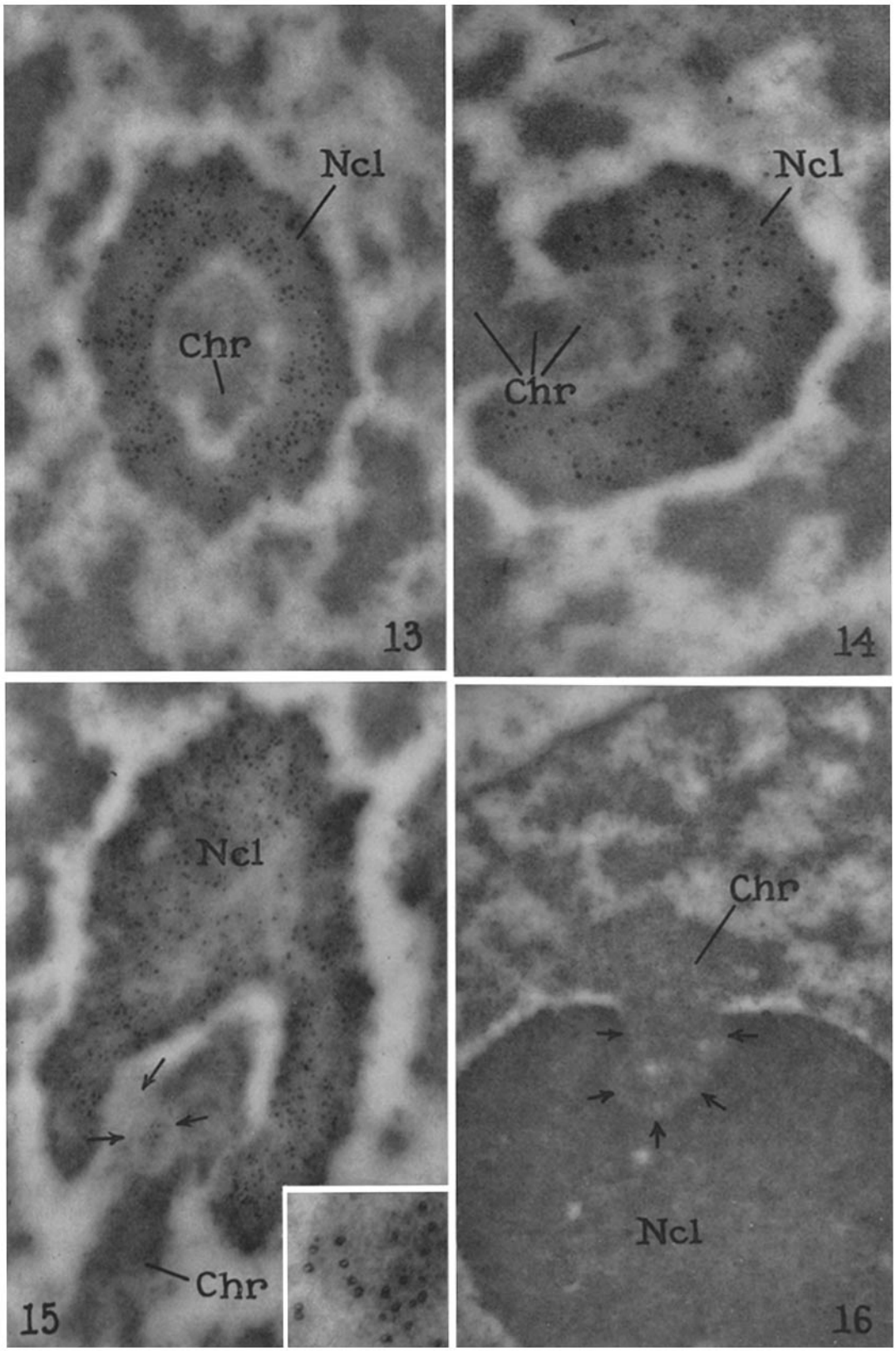
The nucleolus is ring-shaped in cross-section and completely surrounds the chromatin material. The plane of sectioning appears to be perpendicular to the long axis of the chromatin strand projecting inside the nucleolus. At many places there is very close contact between the nucleolus and this chromatin material. The dense granules are clearly restricted to the nucleolus and are not found in the enclosed chromatin material. Osmium tetroxide fixation. × 42,000.

FIG. 14. An interphase nucleolus (*Ncl*) (*Allium cepa*) similar to the one shown in Fig. 13. Here the plane of sectioning coincides with the axis of the chromatin strand (*Chr*). The intimate association of this strand with the nucleolus is well illustrated and its chromosomal nature clearly demonstrated. Note again the localization of the dense granules within the boundaries of the nucleolus. Osmium tetroxide fixation. × 36,000.

FIG. 15. An interphase nucleolus (*Ncl*) in *Allium cepa*. Here there is very little actual contact between the nucleolus and the chromatin material (*Chr*) except at the bottom (arrows), where part of the nucleolar material with dense granules seems to be embedded in the chromatin strand projecting into the nucleolar cavity. Osmium tetroxide fixation. × 32,000.

The insert shows more clearly the doughnut-like appearance of the dense particles in the nucleolus. × 83,000.

FIG. 16. A similar nucleolar-chromatin association in *Vicia faba*. The nucleolus (*Ncl*) is denser and shows a more compact texture than the chromatin material. In spite of the absence of dense granules within the nucleolus it is nevertheless possible to make out the boundary of the chromatin strand (arrows). Here the nucleolus appears to be more intimately connected to the chromatin strand than it is in similar cases in *Allium cepa*. Osmium tetroxide fixation. × 24,000.



(Lafontaine: Nucleolus in meristematic cells)

PLATE 396

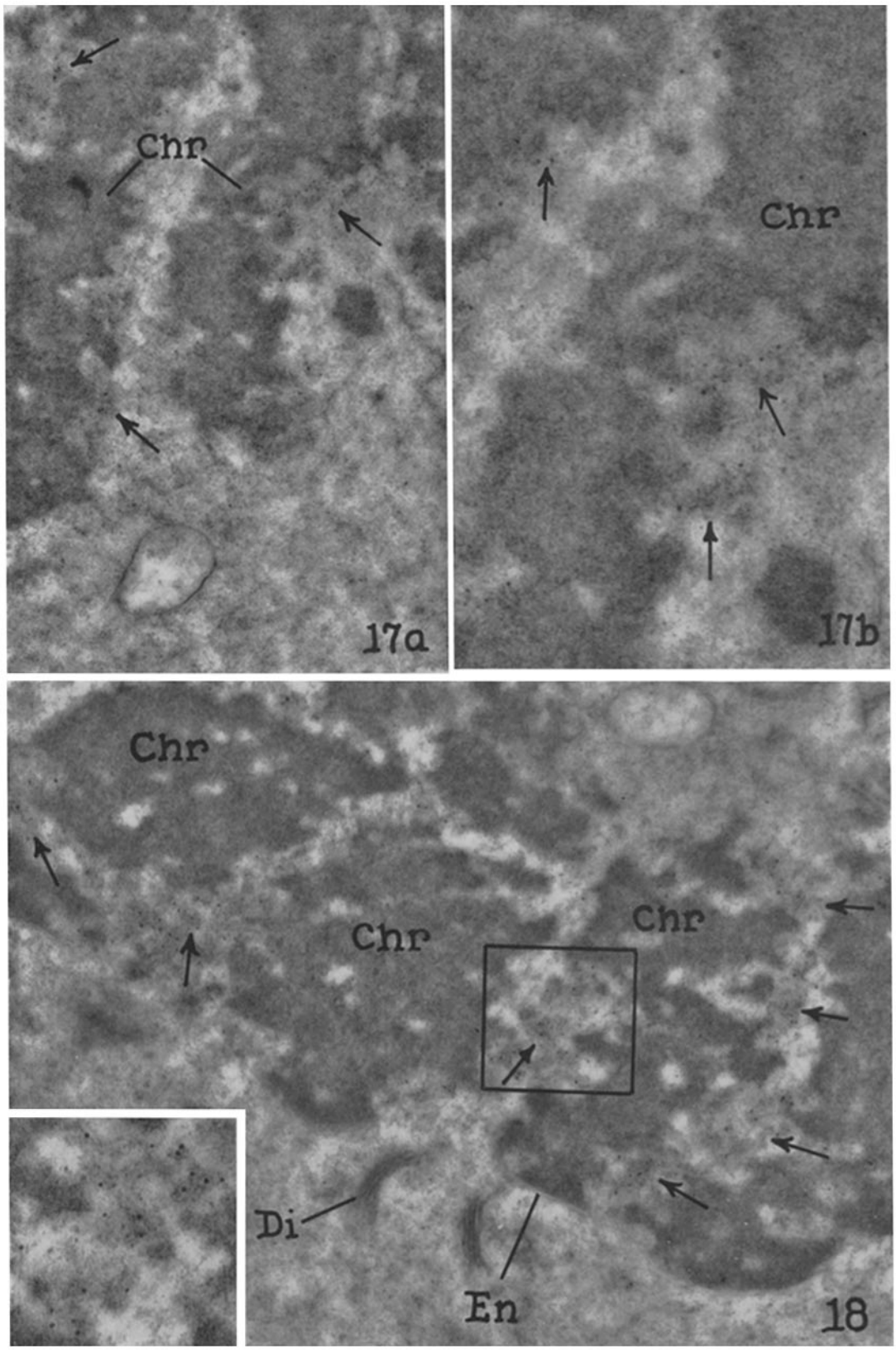
FIG. 17 *a*. Arms of two late anaphase chromosomes (*Chr*) in *Allium cepa*. At several places (arrows) small clusters of dense particles may be observed. Some of them are embedded in material almost matching the chromosomes in density but most clusters are located in low density areas. Osmium tetroxide fixation. × 18,000.

FIG. 17 *b*. Micrograph at higher magnification showing the loose clusters (arrows) of dense granules between the late anaphase chromosomes. Note the relatively low density of the interchromosomal areas. × 33,000.

FIG. 18. Early telophase chromosomes (*Chr*) in *Allium cepa* at the time when the cell plate just begins to form. Here the dense particles have increased in number and are clearly seen between the chromosomes (arrows). They are now embedded in a material which is slightly denser than other regions of the spindle.

A double envelope (*En*) is forming on the outer surface of the chromosomes but it is not yet complete. Note the dictyosomes (*Di*) in the cytoplasm. Osmium tetroxide fixation. × 16,000.

FIG. 18 (insert). Blowup of one of the interchromosomal areas illustrating the distribution of the dense particles. × 26,000.



(Lafontaine: Nucleolus in meristematic cells)

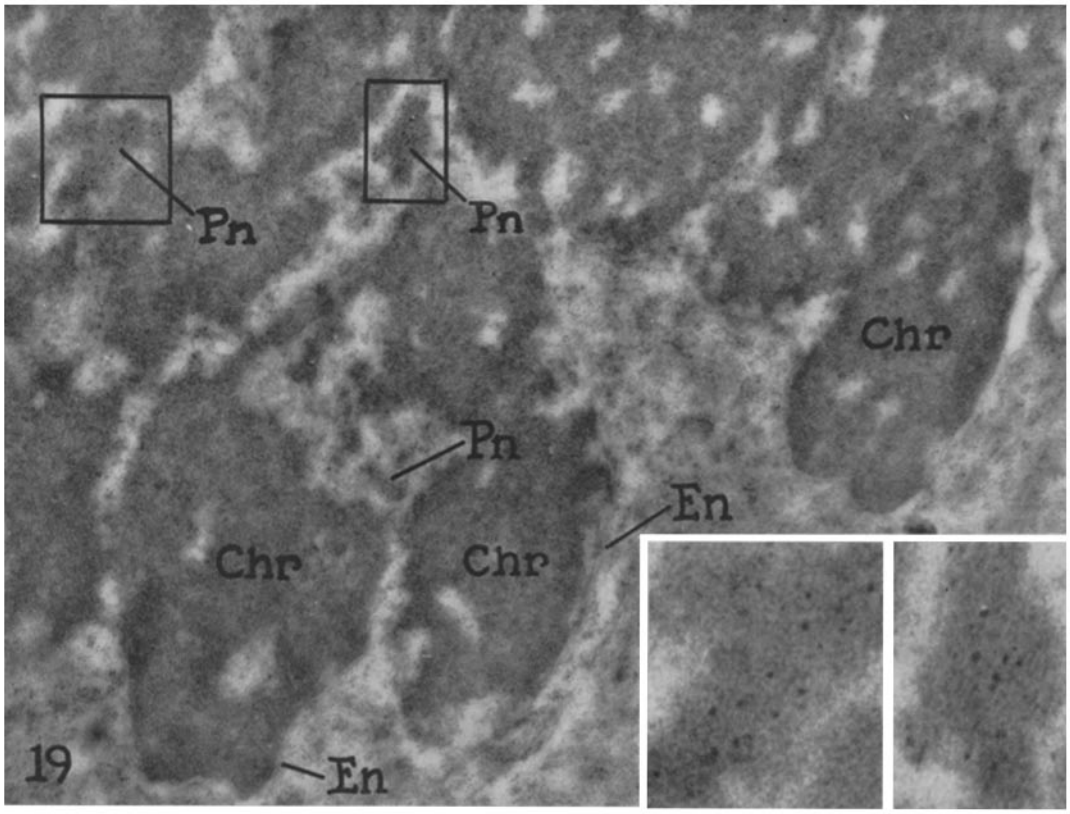
PLATE 397

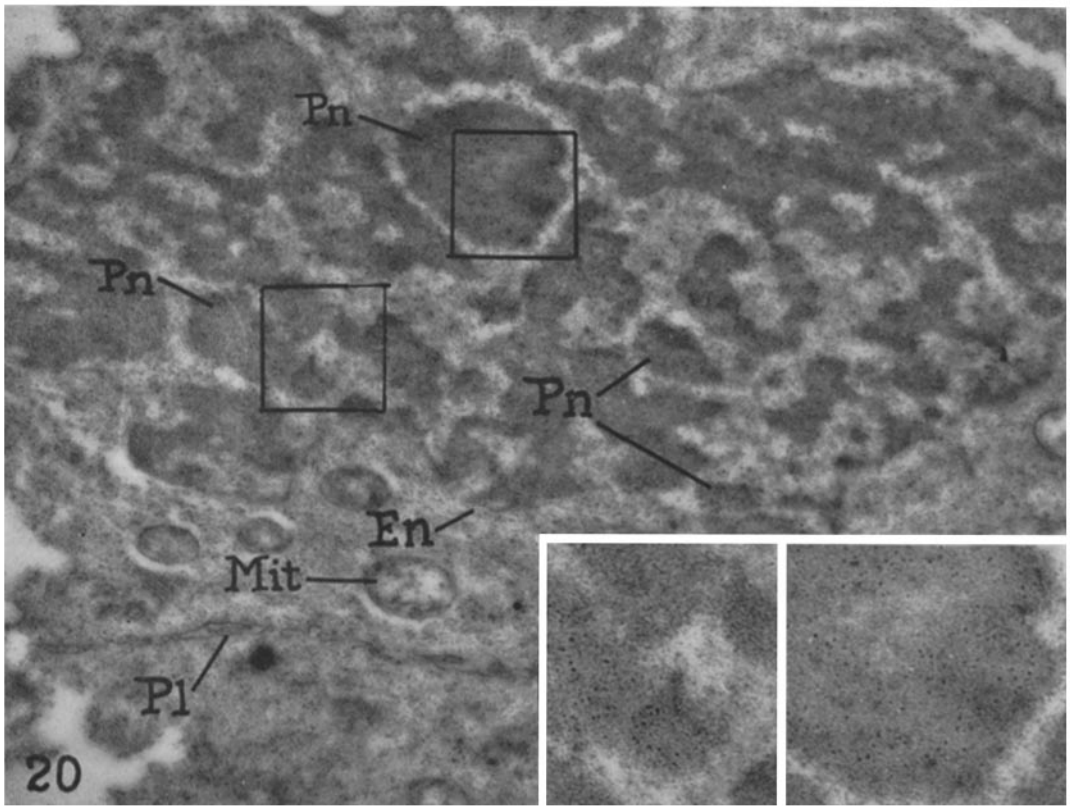
FIG. 19. Part of a more advanced telophase nucleus in *Allium cepa*. The chromosomes (*Chr*) have partly unraveled and the nucleus is surrounded by a more continuous envelope (*En*). The dense granules are now located in small prenucleolar bodies (*Pn*) of various sizes distributed throughout the nucleus. As many as twenty such bodies could be seen in the complete section. Osmium tetroxide fixation. × 28,000.

FIG. 19 (inserts). Blowup of two of the prenucleolar bodies showing the dense granules within them. × 87,000.

FIG. 20. Late telophase nucleus in *Allium cepa*. The vesicles of the cell plate (*Pl*) are fusing together forming a more continuous structure. The nuclear envelope (*En*) appears double and is continuous all around. There are still many prenucleolar bodies (*Pn*) in this nucleus, but they have grown in size. The dense particles are now in much larger quantity and are found in these bodies only. A few mitochondria (*Mit*) may be seen in the cytoplasm. Osmium tetroxide fixation. × 15,000.

FIG. 20 (inserts). Micrographs at higher magnification of two of the prenucleolar bodies illustrating the dense particles. × 32,500.



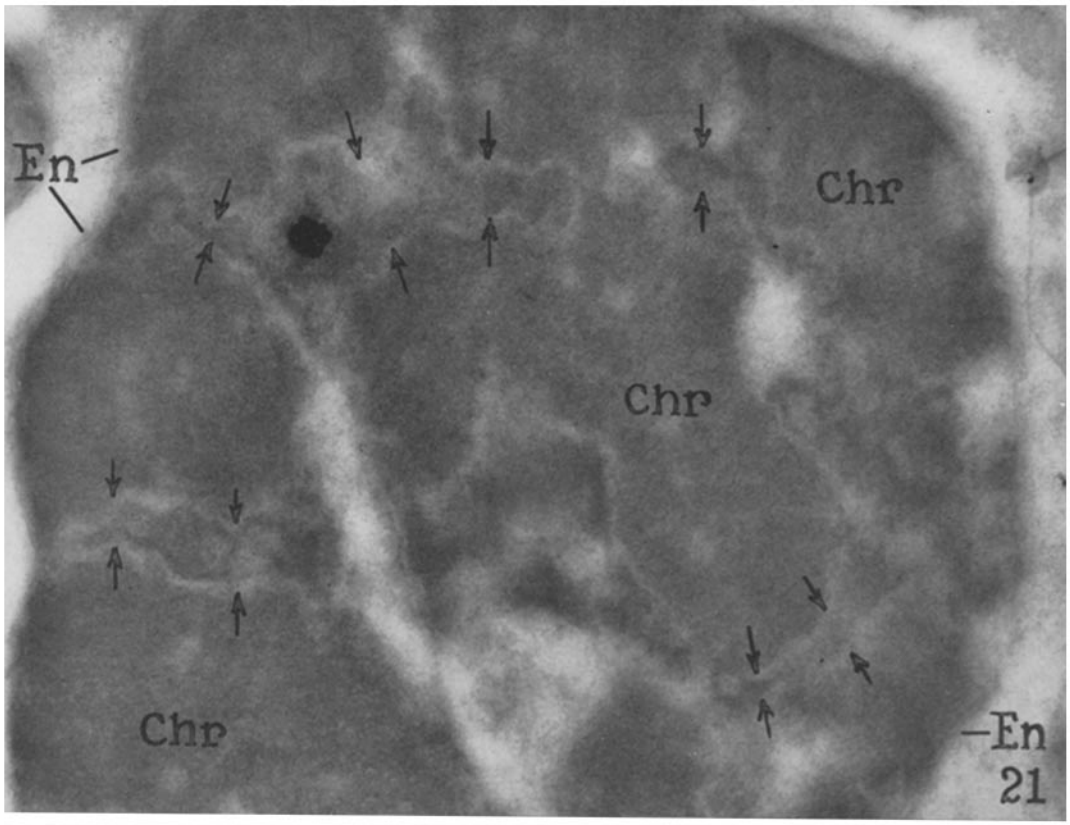


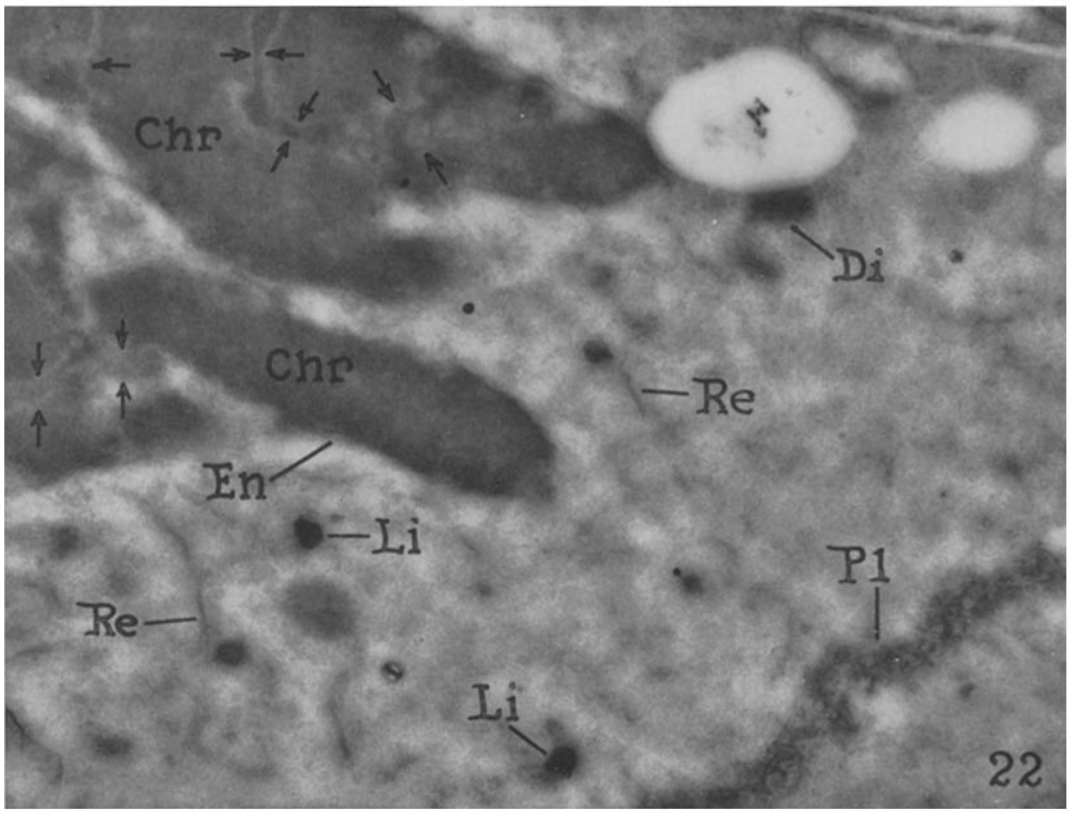
(Lafontaine: Nucleolus in meristematic cells)

PLATE 398

FIG. 21. Early telophase nucleus in *Vicia faba*. The chromosomes (*Chr*) are tightly packed together and a thin layer of prenucleolar material (arrows) is seen between them. The nuclear envelope (*En*) is found in close contact with the chromosomes. Osmium tetroxide fixation. × 24,000.

FIG. 22. Early telophase nucleus in *Vicia faba*, showing prenucleolar material (arrows) between the chromosomes (*Chr*). Note how closely the nuclear envelope (*En*) follows the contours of the chromosomes. The small vesicles on the cell plate (*Pl*) have not yet fused. A dictyosome (*Di*) and elements of the endoplasmic reticulum (*Re*) may be seen in the cytoplasm. The dark bodies probably represent lipide inclusions (*Li*). Osmium tetroxide fixation. × 13,000.





(Lafontaine: Nucleolus in meristematic cells)