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Changes in the Frequency of Two Types of Nuclear Body During the Interphase of Meristematic Plant Cells

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Summary

After impregnating root meristems with silver nitrate two types of small (< 1 μm diameter) body can be seen in the nuclei. These have been termed "dense body" (DB) and "nucleolus-associated body" (NAB). The number of these bodies within a nucleus varies from species to species, but in general DBs are relatively numerous and lie in the nucleoplasm, while the NAB is usually solitary and lies on the surface of the nucleolus. Using nuclear volume as an indicator of the age of the nucleus since mitosis, the numbers of DBs and NABs were related to the nuclear growth cycle. In the meristem of *Pisum sativum* and *Zea mays* DBs are characteristically present in early interphase; in some regions they persist in the nucleoplasm until the next mitosis, in other regions they disappear during interphase. DBs are probably pieces of the pellicle of ribonucleoprotein that coats mitotic chromosomes which have not coalesced (as does the remainder of the pellicle) to form the nucleolus at the start of interphase. NABs grow out from the nucleolar surface during the later stages of interphase. At the end of interphase there is on average 1 NAB per nucleolus.

Keywords: Allium cepa; Brassica napus; Nuclear bodies; Mitotic cycle; *Pisum sativum; Zea mays.*

1. Introduction

The general structure of the cell nucleus is quite well known at the levels of resolution afforded by the light and electron microscopes (LAFONTAINE 1968, JORDAN *et al.* 1980). Nevertheless, the nuclei of plants and animals contain bodies whose structure and function are still imperfectly known [see reviews by LAFONTAINE (1968), BOUTEILLE *et al.* (1982), WILLIAMS *et al.* (1983)]. Two such bodies can be readily seen by light microscopy in meristematic nuclei of certain plant species following silver impregnation. They have been called "dense body" (DB) and "nucleolus-associated body" (NAB) or "karyosome" (BARLOW 1981 a)[1] . Their structure, as seen in root apices of *Pisum sativum* and *Zea mays*, has been described in previous publications (BARLOW 1981 a, 1983) and more details are given in the paper by WILLIAMS *et al.* (1983). Here I describe changes in the frequency of these two bodies during the intermitotic period in roots of these and two other species. The observations give an indication of their possible origin and fate.

2. Materials and Methods

Apices of primary roots, 1-4 cm long, from seedlings of *Brassica napus* cv. Primor, *Pisum sativum* cv. Sprite and *Zea mays* cv. LG 11, and also apices of adventitious roots of similar length on bulbs of *Allium cepa*, were fixed and impregnated with $AgNO_3$ as described by RISUEÑO *et al.* (1973). For light microscopy, the silver-impregnated apices were dehydrated in a graded ethanol series, embedded in Tissuemat and sectioned longitudinally at 16 μm (11 μm in the case of *B. napus*). This thickness ensured many whole, uncut nuclei in each section and a minimum overlap of nuclei. After dissolving the wax in xylene, the sections were mounted under a coverslip in neutral Canada Balsam. For transmission electron microscopy the apices were handled as described earlier (BARLOW 1983).

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[1] In earlier publications (BARLOW 1981 a, b) the dense body was also refered to as an argyrophilic intranuclear body or AIB.

Electron microscopy of silver impregnated material was performed using thin (silver-gold) sections in an AEI EM 6 operating at an accelerating voltage of 80 kV. The sections were stained with uranyl acetate and lead citrate. Some material was also prepared according to the technique of HAWES *et al.* (1981) for viewing in a high voltage electron microscope operating at either 200 or 1,000 kV.

Nuclei in well-defined zones of the silver-impregnated root apices were examined with a Zeiss Photomicroscope II using a x 100 planapochromatic objective lens. The number of DBs and NABs was counted in each nucleus. The major and minor diameters of these nuclei, as well as the diameter(s) of their nucleolus (or nucleoli), were measured using a calibrated filar micrometer. Only uncut nuclei were considered. In some zones of the apex the nuclei and their nucleoli were elongated in the direction of the cell (root) axis. Where the diameter in an axial direction, D_1, was on average appreciably greater than the diameter at right angles to it, D_2, the nuclei were regarded as prolate spheroids and their volume, V, was calculated according to the formula $V = \frac{\pi D_1 (D_2)^2}{6}$. In other zones where D_1 and D_2 were on average approximately equal, the nuclei were regarded as "near-spheres" and their volume calculated according to the formula

$$V = \frac{\pi D_1 D_2 (D_1 + D_2)}{12}.$$

Autoradiography was performed on Feulgen-stained sections of roots of *P. sativum* and *Z. mays* that had been fed with methyl-^{3}H-thymidine (74 kBq/ml) for 40 minutes immediately before fixation in FPA (formalin, propionic acid, 50% ethanol, 1:1:15 v/v/v). The sections were cut at 14–16 μm because whole nuclei were required for observation. After the autoradiographic film (Kodak AR 10) had been developed, fixed and dried, the relative DNA content of both the labelled and unlabelled nuclei was estimated using a Vickers M 85 integrating microdensitometer with the scanning light beam set at 565 nm. The period for which the film was exposed was adjusted to give a grain density over the labelled nuclei that was sufficiently low to have an insubstantial effect on the DNA absorption reading.

3. Results

3.1. The Appearance of Dense Bodies and Nucleolus-Associated Bodies

Only a brief description of DBs and NABs, and of their disposition within the nucleus, will be given here in order to provide the necessary background for the results that follow. A more detailed description of their ultrastructure is given in the accompanying paper by WILLIAMS *et al.* (1983). The DB is spherical (up to about 0.5 μm in diameter) and lies within the nucleoplasm (Fig. 1). Where there are many DBs in a nucleus they show no clustering nor any preferred location within the nucleoplasm. The NAB also has a spherical shape (up to 1 μm in diameter) and is almost always in contact with the nucleolus (Fig. 2). In the few nuclei where it does not contact the nucleolus it lies in the nucleoplasm and can be distinguished from a DB by virtue of its larger size and more translucent appearance. Electron microscopy confirms the contact between NAB and nucleolus (Figs. 2 *b*, *c*). It is important to emphasise that the NAB is quite distinct from nucleolus-associated dense chromatin (the nucleolus organizer region) which also contacts the nucleolar surface (Figs. 2 *b*, *c*).

3.2. Relationship Between the Number of Intranuclear Bodies and the Intermitotic Period of Nuclear Growth

In the results and discussion which follow it is a basic assumption (support for which is presented below) that nuclear volume is related to the age of the nucleus following its birth at mitosis and hence indicates its relative position in the intermitotic period (WOODARD *et al.* 1961, MITCHELL and VAN DER PLOEG 1982). Thus, the smallest nuclei correspond to those that have most recently entered interphase while the largest are those closest to entering mitosis. The silver-staining reveals enough detail of chromatin structure to support this: the smallest and largest nuclei were often recognizably at late telophase/early interphase and prophase, respectively. The relationship between nuclear volume and interphase was verified in closely defined meristematic regions using a combination of autoradiography and microdensitometry (Fig. 3). The unlabelled nuclei fall into two classes with 2 C and 4 C DNA contents; these lie at the small and large ends, respectively, of the range of nuclear volumes. Labelled nuclei have a range of intermediate volumes. Moreover, the small unlabelled (2 C) nuclei reside in shorter cells than the larger unlabelled (4 C) nuclei (data not shown). Therefore, nuclear volume may be taken as a guide to nuclear age. The volume of each nucleus in defined regions of the silver-impregnated root apices was calculated. Then, the volumes were listed in order of increasing size together with the estimates of total nucleolar volume, volume of chromatin (*i.e.*, nuclear volume minus total nucleolar volume), as well as the number of DBs and NABs present in each nucleus. The results presented in Fig. 4 are for three regions of the apex of *P. sativum*: the quiescent centre (QC – which is a slowly proliferating group of cells sandwiched between the proximal surface of the root cap and the distal surface of a central column of cells of the root proper), the stele meristem 350 ± 50 μm above the QC, and the stele 1,200 ± 50 μm above the QC. This latter zone lies near the proximal limit of the stele meristem.

The presentation of these results can be simplified by calculating the mean number of DBs and NABs within nuclei of a set of size-classes spanning the complete range of volume (Fig. 5). Comparable results are shown

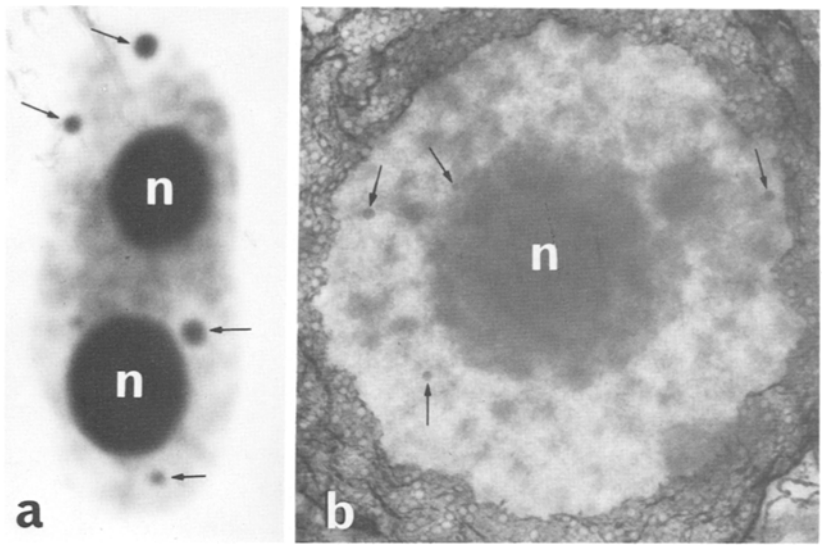

Fig. 1. Dense bodies. *a* Light micrograph of DBs (arrowed) in a silver-impregnated meristematic cell nucleus of *P. sativum*. There are two nucleoli (*n*). × 5,760. *b* High voltage (1,000 kV) electron micrograph showing four small bodies (arrowed) (counterparts of the DB seen with the light microscope) in a 1 μm thick section of a meristematic cell nucleus of *Z. mays*; one of them lies very close to the nucleolus (*n*). × 14,800

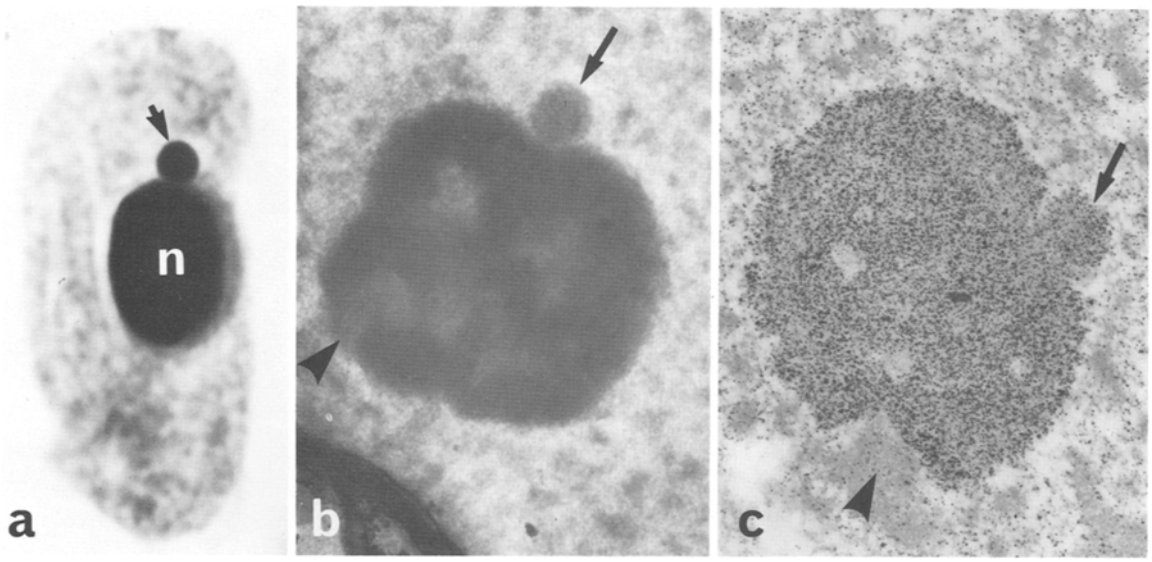

Fig. 2. Nucleolus-associated bodies. *a* Light micrograph of a silver-impregnated nucleolus (*n*) and its NAB (arrowed) in the differentiating stelar zone of *Z. mays*. × 3,840. *b* High voltage (200 kV) electron micrograph of a NAB (arrowed) in the nucleus of a root cap cell of *Z. mays*. Chromatin of the nucleolus organizer region (arrowhead) lies at the opposite side of the nucleolus to the NAB. × 12,000. *c* Electron micrograph (80 kV) of a silver-impregnated nucleus in the quiescent centre of *Z. mays*. As in *b*, the NAB (arrowed) is quite distinct from the chromatin of the nucleolus organizer region (arrowhead). × 20,300

for three zones of the meristem of *Z. mays* (Fig. 6) and for the stele meristem of *B. napus* and *A. cepa* (Fig. 7). The latter species, however, does not possess a NAB.

In all regions of the roots examined the mean number of DBs (Figs. 5–7), and the frequency with which DBs are found (see Fig. 4), is high in small nuclei that are at an early stage of the intermitotic period. The mean may then decline as the nuclei enlarge and approach mitosis. Since the volume of chromatin rises during the intermitotic period in parallel with the total nucleolar volume (see Fig. 4) the decline in the number of DBs is not the result of their obscuration by the growing nucleolus. In *P. sativum* the stele at 350 μm is somewhat different from the other meristematic zones examined in that the mean number of DBs remains approximately constant (at a value of 7–8) throughout most of

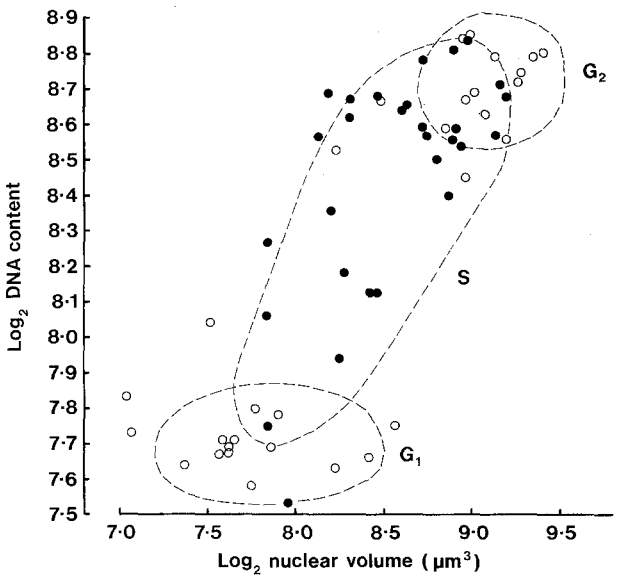

Fig. 3. Relationship between nuclear volume, DNA content (arbitrary units) and ^{3}H-thymidine labelling ability. Unlabelled nuclei are denoted by open symbols, labelled nuclei by closed symbols. The unlabelled nuclei fall into two groups: one group, the G_1 nuclei, have small volumes and low DNA content; the other group, the G_2 nuclei, have larger nuclei and a DNA content twice that of the G_1 nuclei. Labelled S phase nuclei lie between these two groups. The data are from nuclei sampled in the stele 350 μm of one root of *Z. mays*. Comparable results were obtained from other roots

interphase, but shows a marked drop in the largest nuclei examined (Figs. 4 and 5). The mean number and frequency of DBs also remains approximately constant throughout interphase in the stele meristem of *A. cepa*.

The frequency and mean number of NABs per nucleus increase as the nucleus enlarges (Figs. 4–7). NABs tend to be absent in early interphase nuclei but were often seen in early prophase nuclei. Nuclei with two nucleoli are quite common in *P. sativum* and *Z. mays* and each nucleolus may have a body associated with it. Some single nucleoli, particularly in relatively large nuclei, may bear up to three NABs (Fig. 4).

A NAB can be recognized with certainty only if it projects beyond the observed perimeter of the nucleolus; a NAB on the nucleolar surface facing towards or away from the observer may be unrecognized. It follows that the frequency with which a NAB is observed is influenced by the size of both the nucleolus and the NAB. Assuming that a NAB can be positioned at random on the nucleolar surface and that its size is small compared to the nucleolus, then as the nucleolus grows during interphase the frequency of observing a NAB will decline. This clearly cannot account for the finding (Figs. 4–7) that their observed frequencies in-

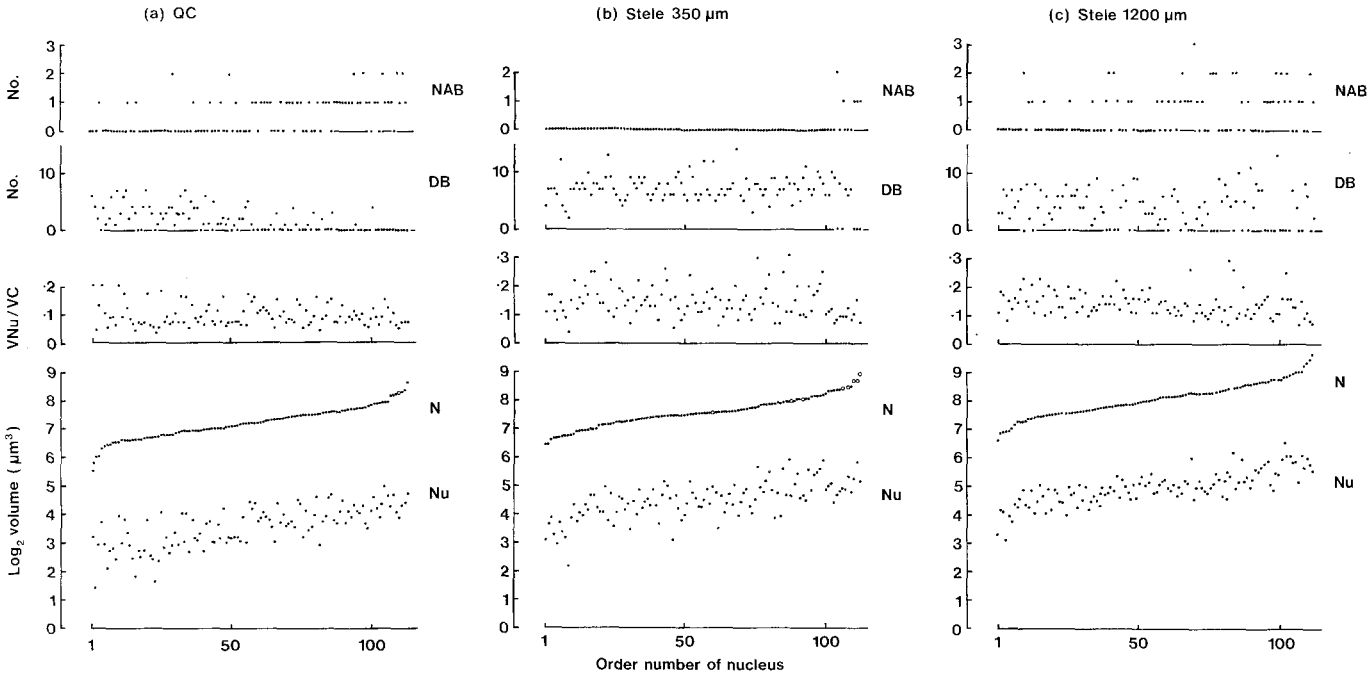

Fig. 4. The number of DBs and NABs, the ratio between nucleolar and chromatin volumes (*VNu/VC*), and total nucleolar (*Nu*) volume in each nucleus (*N*) of three regions of the apex of *P. sativum*: *a* quiescent centre, *b* stele 350 μm, and *c* stele 1,200 μm. The data are plotted in an order determined by the volumes of the nuclei in the sample. Prophase nuclei are denoted by an open symbol, the remainder are in interphase. 112 nuclei were scored in each region

P. sativum

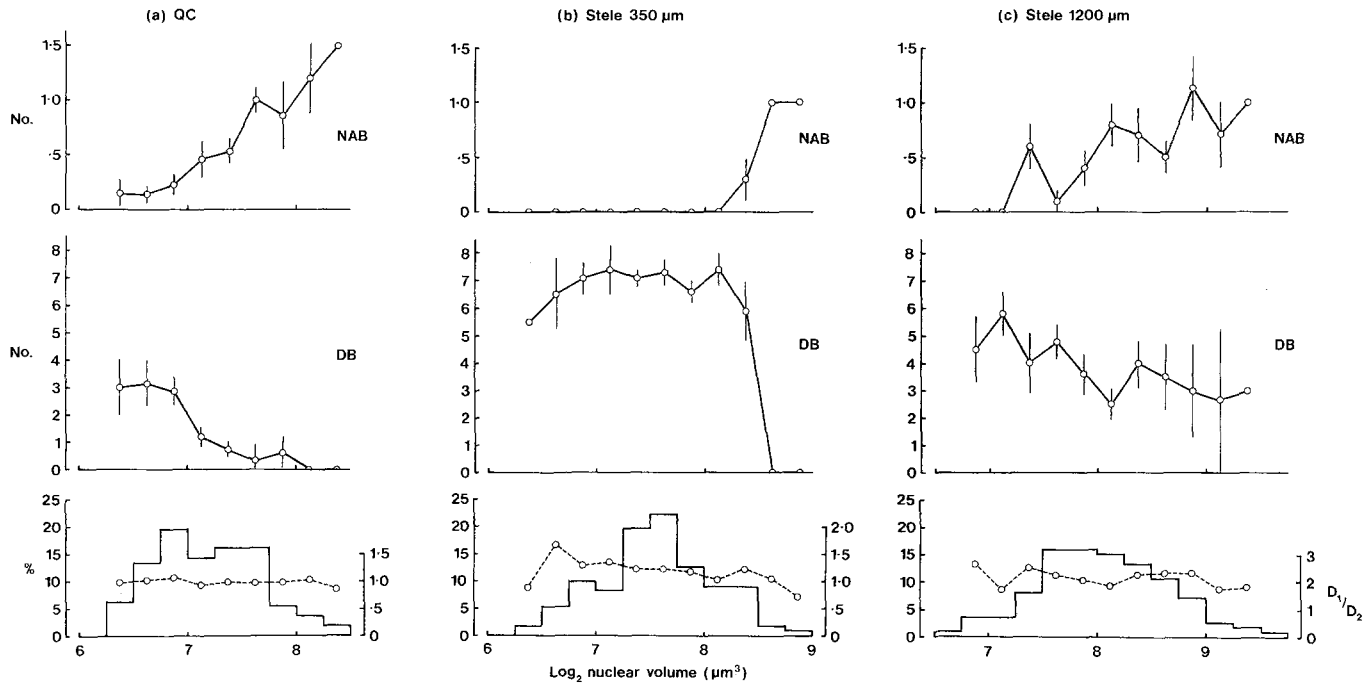

Fig. 5. The same data as Fig. 4 but the nuclei were apportioned into classes according to their volumes [the histogram in the bottom panel shows the frequency (%) of nuclei in each volume class]. The mean number (± SE) of DBs and NABs in each nuclear volume class is recorded in the upper two panels. In the bottom panel the ratio of the two nuclear diameters measured (D_1/D_2) is also given (open symbol and broken line); this ratio determines whether the nuclei are considered as prolate spheroids or as near spheres in calculating their volume. *a* quiescent centre (near sphere), *b* stele 350 μm (prolate spheroid), *c* stele 1,200 μm (prolate spheroid)

Z. mays

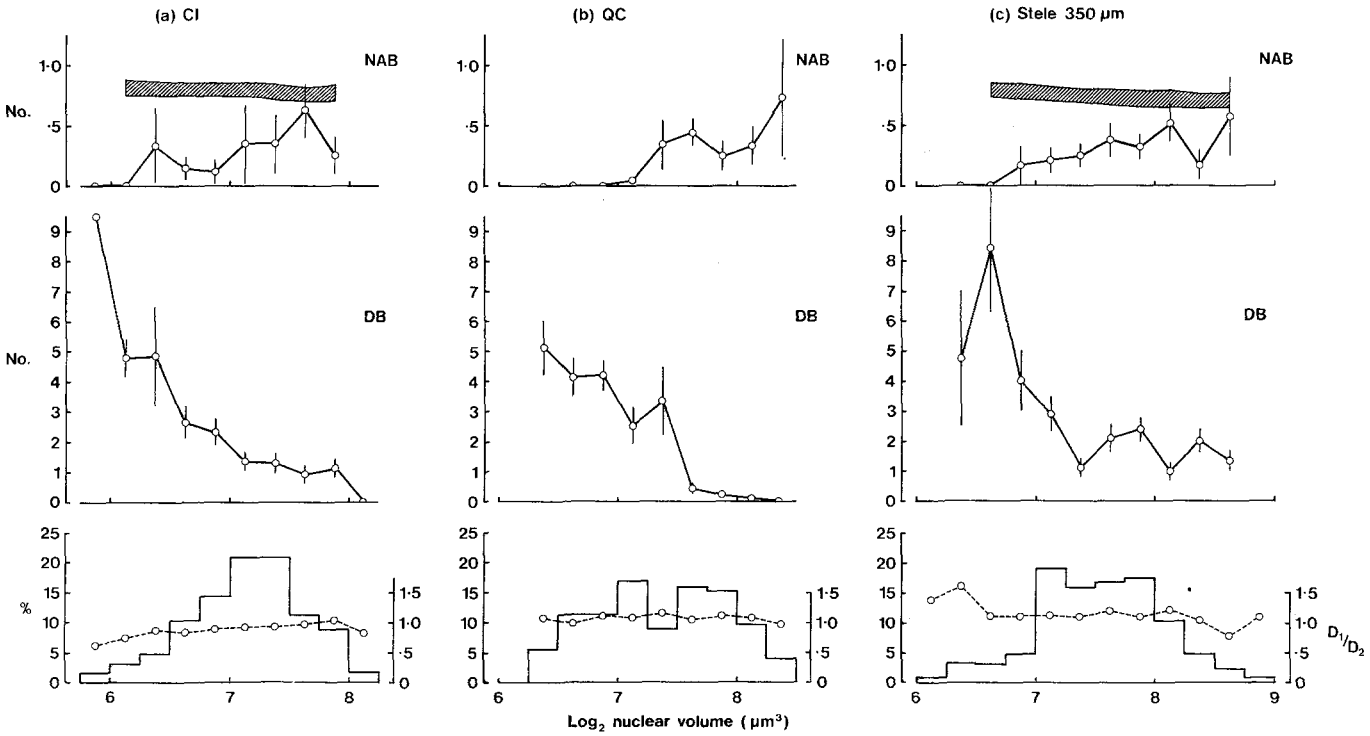

Fig. 6. Data for three regions of the apex of *Z. mays* calculated and plotted in the same way as those in Fig. 5. *a* cap initials (near sphere), *b* quiescent centre (near sphere), *c* stele 350 μm (prolate spheroid). In two of the zones a predicted frequency of NABs is indicated (hatched area in the upper panel – see text for details of its derivation). 125 nuclei were scored in each region

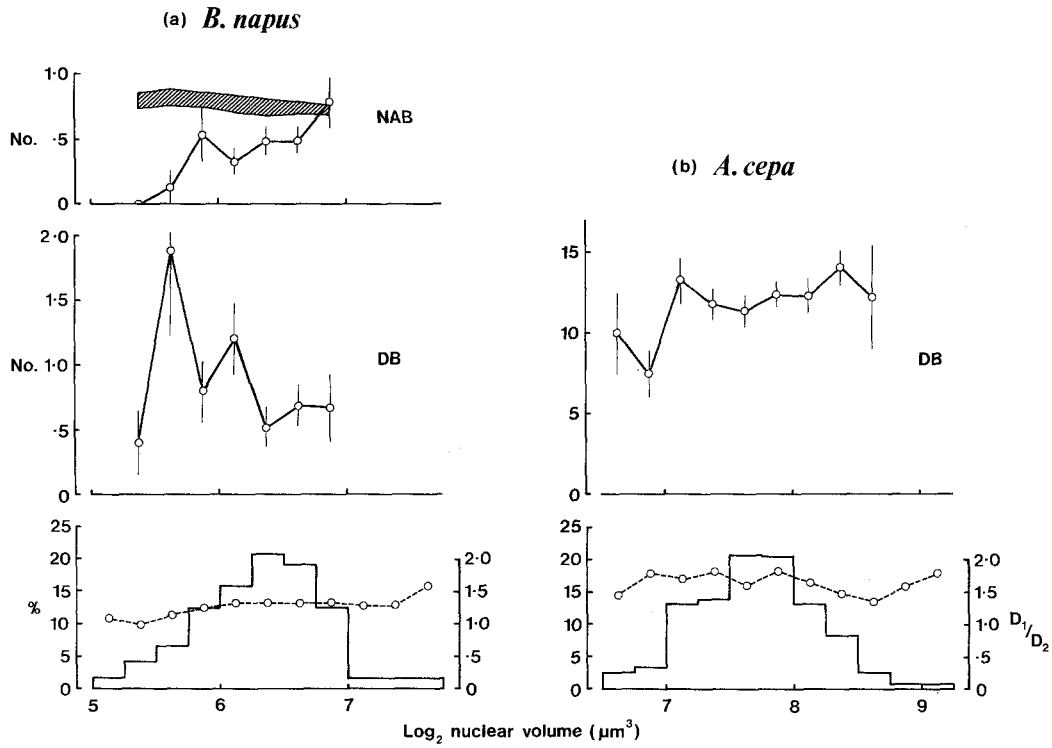

Fig. 7. Data for the stele meristems of *a B. napus* and *b A. cepa* prepared in the same way as those in Fig. 5. *A. cepa* does not possess a NAB. A predicted frequency of NABs (hatched area) is shown for *B. napus*. 120 nuclei were scored in *B. napus*, 121 in *A. cepa*

crease. The probability, P, of observing a NAB, given the above assumptions, can be calculated according to the formula

$$P = \frac{2\sqrt{d\,\delta}}{d + \delta}$$

where d is the diameter of the nucleolus and δ is the diameter of the NAB. For a spheroidal nucleolus this formula was used with $d = \sqrt{d_1\,d_2}$ as an approximation, d_1 and d_2 being the observed major and minor diameters. Using 0.6 and 1.0 μm as the lower and upper limit of δ, and the actual mean diameters of the nucleoli in each size class of nuclei, the probabilities so calculated for various zones of the roots are shown in Figs. 6 *a, c* and 7 *a*. Note that the probable frequency of observing a single NAB on a nucleus approaches the observed frequency as the nuclei enlarge. This indicates that a nucleolus bears on average one NAB by the end of interphase. (It should also be noted that the probabilities are calculated for a single nucleolus, while the observed frequencies apply to a population of nuclei in which some nuclei have two nuclei. However, the frequency of binucleolate nuclei in these populations was low: it was 7 nuclei out of 125 in the stele 350 μm, 18 out of 125 in the cap initials of *Z. mays*, and 3 out of 120 in the stele meristem of *B. napus*. NABs were seen in only 2 of the binucleolate nuclei, all the others were in mononucleolate nuclei.)

In some nuclei, particular those at prophase, a NAB was found free in the nucleoplasm. This observation suggested that the NAB initially might grow from the body of the nucleolus, then later detach from it. Evidence for this was sought in the QC of *P. sativum* where NABs are particularly prominent. NABs were scored as "early" if a small projection could be seen on the nucleolus, as "late" if a large projection could be seen, and as "detached". These three stages, when matched against nuclear volume, should follow each other if the behaviour of the NAB is as proposed above. The results (Fig. 8) indicate that there is such a pattern of NAB behaviour. Detached NABs occurred in 9.2% of the 184 interphase nuclei examined in which a NAB was present; however, out of 17 prophase nuclei 12 (70%) contained a detached NAB.

Inspection of results such as illustrated in Fig. 4 indicates a negative interrelationship between DBs and NABs. That is, in many regions of the apex of *P. sativum* and *Z. mays* DBs are present in nuclei from

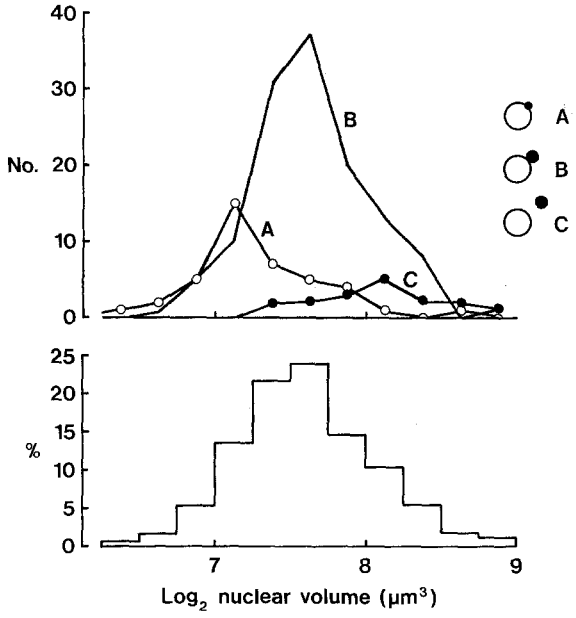

Fig. 8. Frequency of attached [early (*A*) and late (*B*)] and detached (*C*) NABs (upper panel) in nuclei of different volume classes (their frequency is shown in the lower panel) in the quiescent centre of *P. sativum*. 184 nuclei were scored

which a NAB is absent; and conversely, NABs are often present when DBs are absent (Table 1). However, this relationship seems less good in the stele meristems *B. napus* or *Z. mays*; in both these zones DBs and NABs frequently co-exist within the same nucleus.

3.3. Electron Microscopy and the Origin of Dense Bodies

Electron microscopy of silver-impregnated material helps to elucidate the origin of the DBs and confirms images seen with the light microscope. Metaphase chromosomes are covered by argyrophilic material, but at late telophase this material separates from the chromatin and coalesces (Fig. 9 *a*) to form the new nucleolus at early interphase (Fig. 9 *b*). Small pieces of the argyrophilic material persist in the nucleoplasm lodged amongst the clumps of chromatin (Fig. 9 *c*) and give rise to the DBs recognizable with both the electron and light microscopes (Figs. 1 and 9 *d*).

4. Discussion

On the basis of the behaviour of the argyrophilic material during mitosis and the way in which the numbers of DBs and NABs change during interphase, a fairly detailed interpretation can be offered for the origin and fate of these two bodies.

Chromosomes at mitosis are coated with a pellicle of ribonucleoprotein (RNP) that has affinity for silver (MORENO DÍAZ DE LA ESPINA *et al.* 1976) and which derives from the nucleolus that disintegrated at the preceeding prophase. During late telophase this RNP pellicle segregates from the chromosomes and, as MORENO DÍAZ DE LA ESPINA *et al.* (1976) and STOCKERT *et al.* (1970) suggest, coalesces to form one or more nucleoli. Certain portions of this RNP, however, are not incorporated into the reforming nucleoli and remain in the nucleoplasm where they are recognized as DBs (Figs. 9 *c*, *d*). Consistent with this interpretation are the observations of HÅKANSSON and LEVAN (1942) on the disparate behaviour of pre-nucleolar material on the telophase chromosomes of *Pisum sativum*. Similar

Table 1. *Frequency of dense bodies (DB) and nucleolus-associated bodies (NAB) in different regions of the root apices of Zea mays and Pisum sativum.* + denotes presence; − denotes absence

Frequency (%)								
Region[a]	*Z. mays*				*P. sativum*			
		DB				DB		
	NAB	+	−	n	NAB	+	−	n
Cap initials	+	6.7	16.4	134	+			
	−	66.4	10.4		−	not scored		
Quiescent centre	+	3.1	19.7	130	+	1.6	42.8	182
	−	61.5	24.6		−	46.2	9.3	
Stele 350	+	14.1	21.7	185	+	0.5	1.1	185
	−	46.2	17.9			98.4	0	
Stele 1,200	+	9.0	47.0	200	+	5	34.0	162
	−	27.0	17.0		−	59.2	1.2	

[a] Number refer to distance (μm) from the cap-quiescent centre junction.

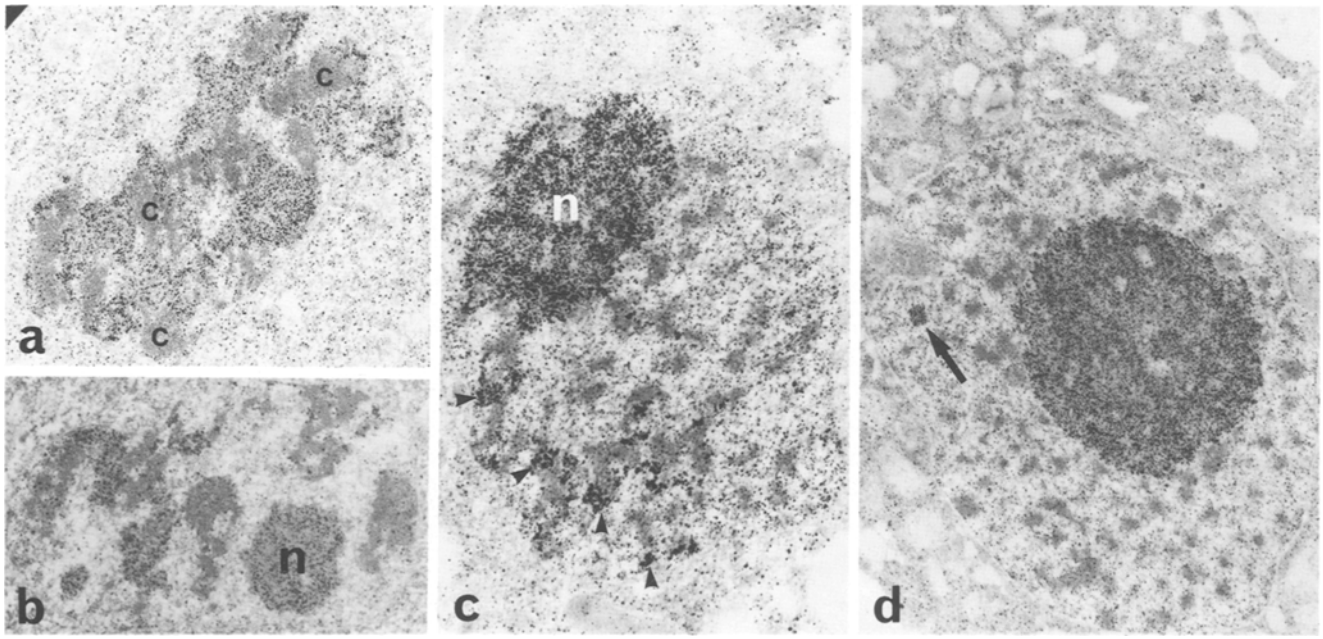

Fig. 9. Electron micrographs (80 kV) of silver-impregnated nuclei in *Z. mays*. *a* Telophase. Note argyrophilic material between the chromosomes (*c*). ×11,550. *b* Slightly later stage than *a*. A reforming nucleolus (*n*) is present. ×14,400. *c* Early interphase. The argyrophilic material has coalesced into the new nucleolus (*n*); small clusters of argyrophilic material (some of which are arrowed) have not coalesced and will probably remain in the nucleoplasm as DBs. ×11,550. *d* Interphase nucleus with a DB (arrowed). ×9,700

findings have also been made with cultured animal cells (PHILLIPS and PHILLIPS 1979, HERNANDEZ-VERDUN *et al.* 1979). The factors determining the coalescence or non-coalescence of pre-nucleolar material are not understood, though the former process (*i.e.*, nucleologenesis) can be accelerated when protein synthesis is inhibited by drugs such as cycloheximide and anisomycin, or delayed by inhibitors of RNA synthesis such as cordycepin (GIMÉNEZ-MARTÍN *et al.* 1974, STOCKERT *et al.* 1970). It is also possible that the structure of the chromatin influences the coalescence of pre-nucleolar material since it is noticeable that nuclei of species with an areticulate nuclear structure contain fewer DBs than nuclei with a reticulate structure (BARLOW 1981 b). Moreover, the acceleration of nucleologenesis induced by adenosine is coupled with a faster rate of chromosome decondensation (GONZÁLEZ-FERNÁNDEZ *et al.* 1982). Another possibility is that the coalescent (nucleolar) and non-coalescent (DB) substances are not identical and so give rise to different structures.

DBs persist to varying degrees across the intermitotic period. In some meristematic regions the mean number of DBs remains roughly constant throughout interphase. By contrast, in slowly proliferating cells such as occur in the quiescent centre, or in cells where the proliferative fraction is less than unity (*e.g.*, stele 1,200 μm), the number of DBs approaches or actually falls to zero well before the nuclei have completed their phase of growth. Given the long period of nuclear growth of these latter populations, two explanations can account for the declining numbers of DBs. One is that the DBs disperse in the nucleoplasm, or at the nuclear membrane, and their constituents pass into the cytoplasm; the second is that they are eventually gathered into the nucleolus. [A third possibility, that can apply only to cells of the quiescent centre, is that because some of the cells have not divided following germination and the commencement of root growth, their nuclei will not have had occasion to acquire DBs, assuming that their origin depends upon mitosis as suggested above. However, DBs have been seen in both light and electron microscope preparations of nuclei of dormant cells (RISUEÑO and MORENO DÍAZ DE LA ESPINA 1979, and personal observation)]. Migration of DBs to the cytoplasm was favoured by RISUEÑO *et al.* (1978). If migration is the only fate of the DBs then the structure of the interphase chromatin may influence this and bear a relationship with the way in which DB numbers change during interphase. A rough calculation can be made of chromatin density on the basis of its volume and DNA content. When applied to the different zones of the roots of the four species studied it is found that

nuclei with more densely packed DNA (*e.g.*, the nuclei of *A. cepa*) show much less decline in DBs across interphase than nuclei with less densely packed DNA (*e.g.*, nuclei in the stele 350 μm of *Z. mays*). However, such a relationship has relevance only if the DBs do indeed migrate across the nucleoplasm and that the chromatin offers a resistance to their movement.

There is also the possibility that DBs not only disperse but also arise "*de novo*" as packages of RNP that "bud" from the nucleolus, or that they arise at some other site of genetic activity. Evidence for a *de novo* origin of DBs during interphase is presented by RISUEÑO *et al.* (1978). They found that the number of argyrophilic bodies in nuclei of *A. cepa* rose from a mean of about 7 (max. of 20) in G_1 to a mean of about 15–17 (max. of 30) in late S and G_2 phases. An increase of this magnitude across interphase in the number of DBs was not found in any of the present material, including *A. cepa*. MORENO DÍAZ DE LA ESPINA *et al.* 1982 a, b) have suggested that these bodies, though not directly synthesising RNA, are involved in processing RNA within the nucleus. Small, spherical intranuclear bodies consisting of RNP have been found in organisms as diverse as the alga *Acetabularia* (WERZ 1962), the fly *Chironomus* (STOCKERT and DÍEZ 1979), and the rat (MONNERON and BERNHARD 1969) and are considered by many of these authors to be carriers of genetic information.

The bodies associated with the nucleolus grow out from the superficial zone of the nucleolus and may even detach from it. Detached NABs are most frequently seen late in the intermitotic period and in prophase; whether detached or not they presumably disperse, along with the nucleolus, at prometaphase. NABs seem to be a prominent feature of slowly or non-proliferating cells (BARLOW 1983), but the reason for this is unclear. It cannot simply be that their formation takes a relatively long period of time since they are a regularly found on the nucleolus of cap initial cells of *Z. mays* and meristematic cells of *B. napus* both of which have an intermitotic period of 12 hours or less.

In many cell types relatively few nuclei contain both DBs and NABs (Table 1). The presence of DBs seems to preclude the presence of NABs or *vice versa*. A reasonable explanation is that each structure is characteristic of a definite portion of the intermitotic period, DBs being typical of early interphase, NABs of late interphase. In some cells, particularly in *Z. mays*, there is a period when neither structure is present. This may correspond to a period before NAB formation but following the dispersion of the DBs. This explanation does not preclude the possibility that the NAB is in some way derived from the DBs. Indeed, the absence of NABs in species such as *A. cepa*, or in regions such as the stele meristem of *P. sativum*, might be accounted for in this way; that is, NABs do not form perhaps because the DBs persist and do not disperse.

The function of the NAB in the life of the cell is unknown. However, the production of small bodies by the nucleolus and their liberation into the nucleoplasm (and in some cases to the cytoplasm) is known in organisms as diverse as the alga *Polytoma* (GAFFAL and SCHNEIDER 1978), the rat (particularly in neurons – HARDIN *et al.* 1969), and plant galls (WOLL 1956). It is worth recalling that WOLL was able to watch, in living cells, the production of these bodies by the nucleolus. Thus, NABs may, in certain circumstances, be a vehicle for the transfer of nucleolar material to the cytoplasm.

It seems unlikely that DBs and NABs, which appear to be quite widespread in many species, and present in an important zone of cell division and differentiation can be entirely superfluous to cellular function.

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