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The Nuclear Pores of Early Meiotic Prophase Nuclei of Plants

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Summary. Pollen mother cells at early meiotic prophase from *Fritillaria lanceolata*, *F. mutica*, *Tulbaghia violacea*, the lily "Formobel", *Triticum aegilopoides*, *T. dicoccoides*, *T. aestivum* and synaptic and asynaptic forms of *T. durum* were studied in thin sections with the electron microscope (a) in relation to distribution of nuclear pores (b) in respect of fine structure of the pore complex in those of the first four. The pores were distributed in random clusters during leptotene to pachytene in all plants, except in the two forms of *T. durum* where there were either no pores or so few that they were not detectable. Probably correlated with this, the two membranes of the nuclear envelope were often widely separated and frequently sacculated. No pores were seen at leptotene in the part of the envelope to which, in the *Fritillarias* and lily, the nucleolus was adpressed at this time. Evidence supporting a recent model which proposes that annuli are composed of three rings of eight granular subunits was obtained. These subunits as well as a dense central element, observed in most pores, were composed of filaments about 3 nm in diameter and evidently protein in character. There was evidence of a continuity between filaments in the central element and those in the rings of subunits which encircle the pore aperture at both the nuclear and cytoplasmic sides of the pore. In profiles of pores knobbed filaments were sometimes seen extending laterally from the pore wall into the perinuclear space at two sides. Questions concerning the role of the annulus are discussed.

Key-Words: Nuclear membrane — Pores — Meiotic prophases — Plant cells.

Introduction

Although there is an extensive literature concerning the structure and distribution of nuclear pores as seen with the electron microscope in nuclei of animal and plant tissues, relatively few such observations have been made in nuclei of cells at early meiotic prophase. The present study is concerned with these aspects in pollen mother cells. The rather irregular shape of the nuclei in these cells particularly during leptotene and zygotene provides a favourable situation for study of pores, since in thin sections the envelope is then cut in planes both perpendicular and substantially parallel to the nuclear surface.

In this paper we use the term "pore" in the generally accepted sense; the term "pore complex" (after Watson, 1959) for the pore and its associated annular material and "annulus" for that part of the pore complex which is seen face-on when in thin sections the envelope is cut almost parallel to the nuclear surface.

Materials and Methods

Pollen mother cells (p.m.c.) at early meiotic prophase were obtained from the following plants: *Fritillaria lanceolata*, *F. mutica*, *Tulbaghia violacea*, the triploid hybrid lily "Formobel", *Triticum aegilopoides*, *T. dicoccoides*, two cultivars of *T. aestiuvm*, *T. durum* and two asynaptic mutants thereof. Cells at pre-meiotic interphase were obtained from anthers of *F. mutica*.

Strings of p.m.c. expelled from anthers cut at one end, or entire anthers as was necessary with the wheats and *T. violacea*, were fixed in ice-cold 10% formalin in phosphate buffer (pH 7.6) plus 0.1% calcium chloride and 0.3% sucrose for 18–24 h. After fixation, the specimens were rinsed a few times in water and then transferred to Caulfield's osmium-sucrose (plus calcium chloride as above) for 2 h, in order to enhance contrast of the image in the electron microscope. To obtain further contrast they were stained 1 h in 2% uranyl acetate in either absolute ethanol or 70% acetone, according to the embedding medium used, as steps in dehydration. Mostly, after rinsing in water the specimens were dehydrated through an ethanol series and embedded in cross-linked methacrylate (Kushida, 1961). A few were dehydrated through an acetone series and embedded in Epon.

Some fixed strings of p.m.c. of the lily, not post-treated with osmic acid, were stained in two different ways. One sample was dehydrated through an ethanol series and then stained 2 h in 2% phosphotungstic acid in absolute ethanol (Gordon and Bensch, 1968), as the last step in dehydration. After staining they were rinsed rapidly in absolute ethanol and embedded in cross-linked methacrylate. Epon embedding was unsatisfactory because of loss of stain. The other sample was stained continuously, in the dark, in an acetone series saturated with uranyl acetate by steps during dehydration (Hills and Plaskitt, 1968). Epon was used for embedding.

Sections were cut with a Cambridge-Huxley microtome. When desirable, the sections were stained in lead citrate (Reynolds, 1963). The micrographs were taken with an Elmiskop 1 a and all measurements made after calibrated magnification, using the catalase method of Wrigley (1968).

Observations

1. Distribution

It will be apparent that only a rough idea of the distribution of pores in the nuclear envelope can be obtained from a study of nuclei in thin sections. Their arrangement and spacing within a group can be determined in face-on views of arrays of annuli, but their absence in any extensive region of the envelope will be detectable only in those stretches cut perpendicular to the nuclear surface.

In all the plants studied, with the exception of synaptic and asynaptic *T. durum*, it was clear that pores were irregularly spread over the nuclear envelope during prophase of meiosis. As is illustrated in Figs. 1 and 2, they were often distributed in clusters, in which they were spaced about 12.5 nm from centre to centre, that were encircled by areas of variable size in which they were widely spaced or did not occur. In some sections the regularity of pore formation suggested that wide differences existed between nuclei in the size of areas devoid of pores. There was, however, no obvious indication that the frequency of pores changed between leptotene and pachytene. In *Tulbaghia* where the sections could be cut so as to include tapetal tissue, there was no evidence of a relationship between the positioning of pores and position of this tissue.

In the p.m.c. of the lily and in those of *F. lanceolata* and *F. mutica*, the nucleolus was invariably tightly pressed against the nuclear envelope during leptotene in the form a cap, as noted also in other lilies (Therman, 1951; Moens, 1968). An examination of the envelope in all these plants at this stage showed that, in the region where the nucleolus was adpressed, pores apparently were not formed (Fig. 3). Again in the *Fritillarias*, there was no regularity in distribution of pores in those parts of the envelope over the typically surface-lying chromocentres (cf. Fig. 4a and b).

The most surprising observations were in the anthers of the synaptic and asynaptic plants of *T. durum*. In the p.m.c. of these, the envelopes appeared to

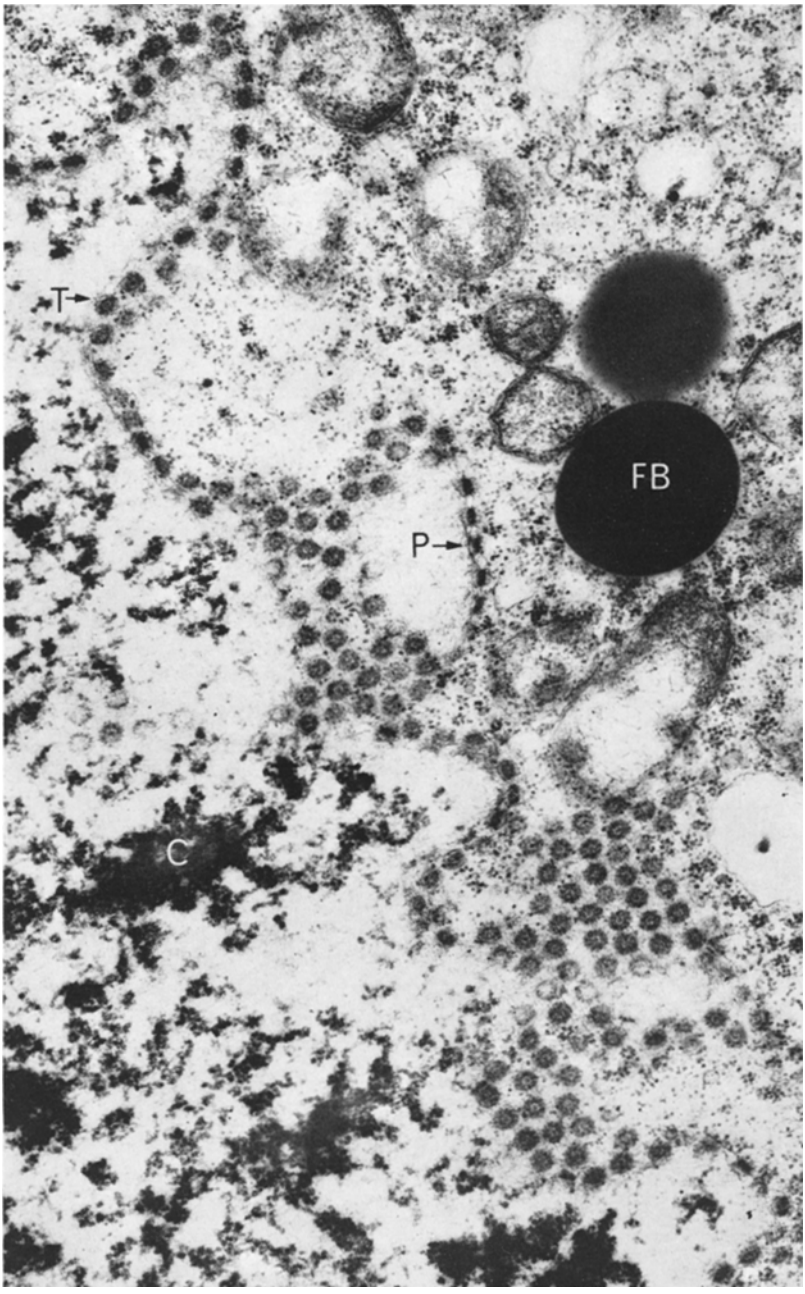


Fig. 1. Micrograph from an Epon section of a p.m.c. at late zygotene of *Fritillaria lanceolata*, showing arrays of annuli face-on and pore complexes in perpendicular (*P*) and tangential section (*T*). Note most annuli have a central plug. *C* chromatin, *FB* fat body, ×30000

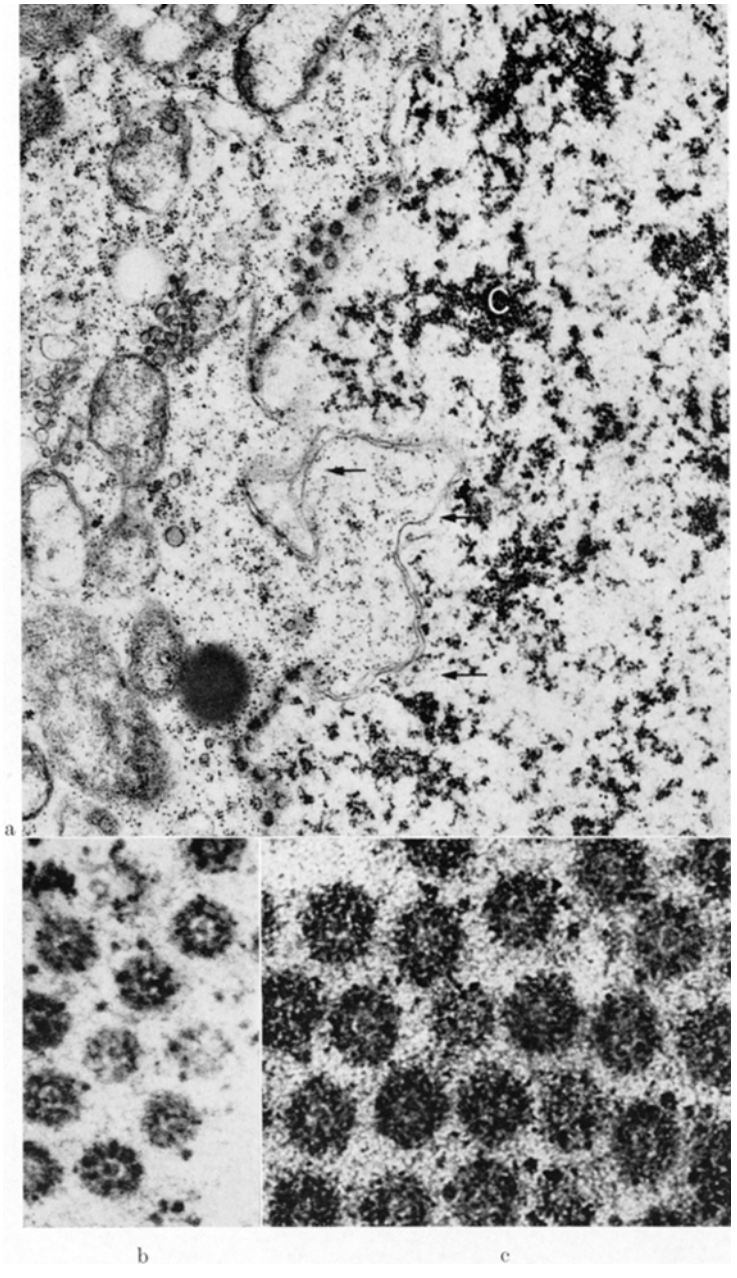


Fig. 2a–c. Micrographs from Epon sections of p.m.c. of *Fritillaria lanceolata* (a) at leptotene with pore complexes in tangential and perpendicular section and as indicated by arrows, stretches of envelope devoid of pores, × 20000 (b) at zygotene with annuli in tangential section, × 80000 (c) at zygotene with annuli face-on, X 100000

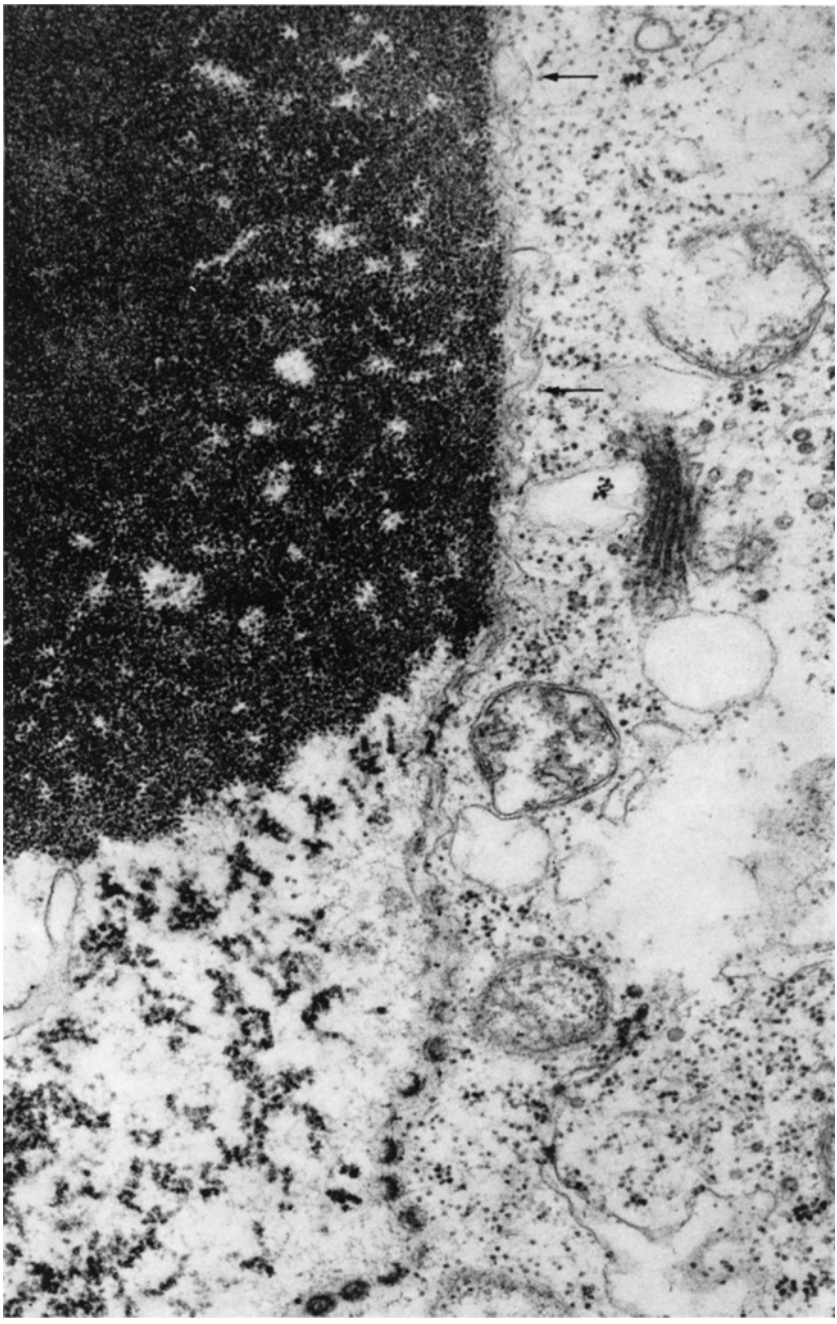


Fig. 3. Micrograph from an Epon section of a p.m.c. at late leptotene of *Fritillaria lanceolata*, showing the nucleolus against the envelope which is tortuous with the two membranes widely separated and devoid of pores in the region where the nucleolus is adpressed; some pore complexes in tangential section are seen below the nucleolus, X 40000

have no pores, or at least so few that they were not detectable. Presumably, because of this the two membranes comprising the envelope were often widely separated and frequently sacculated, particularly the one innermost (Fig. 5). There was no convincing evidence of pinocytosis or reverse pinocytosis at these points. The seeming absence of pores was not confined to one particular phase of prophase, since in anthers of the synaptic plants stages from leptotene to pachytene were examined.

The dearth of pores in the material from *T. durum* prompted a cursory examination of the envelopes of nuclei at zygotene or pachytene of the following: *Triticum aegilopoides*, *T. dicoccoides* and two varieties of *T. aestivum*. In all of these the distribution of pores was in random clusters.

There was a lack of synchrony amongst the anthers in a flower bud of *F. mutica* which made it possible to study the envelope of nuclei at pre-meiotic interphase. The distribution of pores in these envelopes appeared to closely follow those of the meiotic prophase nuclei, there were areas of variable size in which pores were not formed.

2. Structure of the Annulus

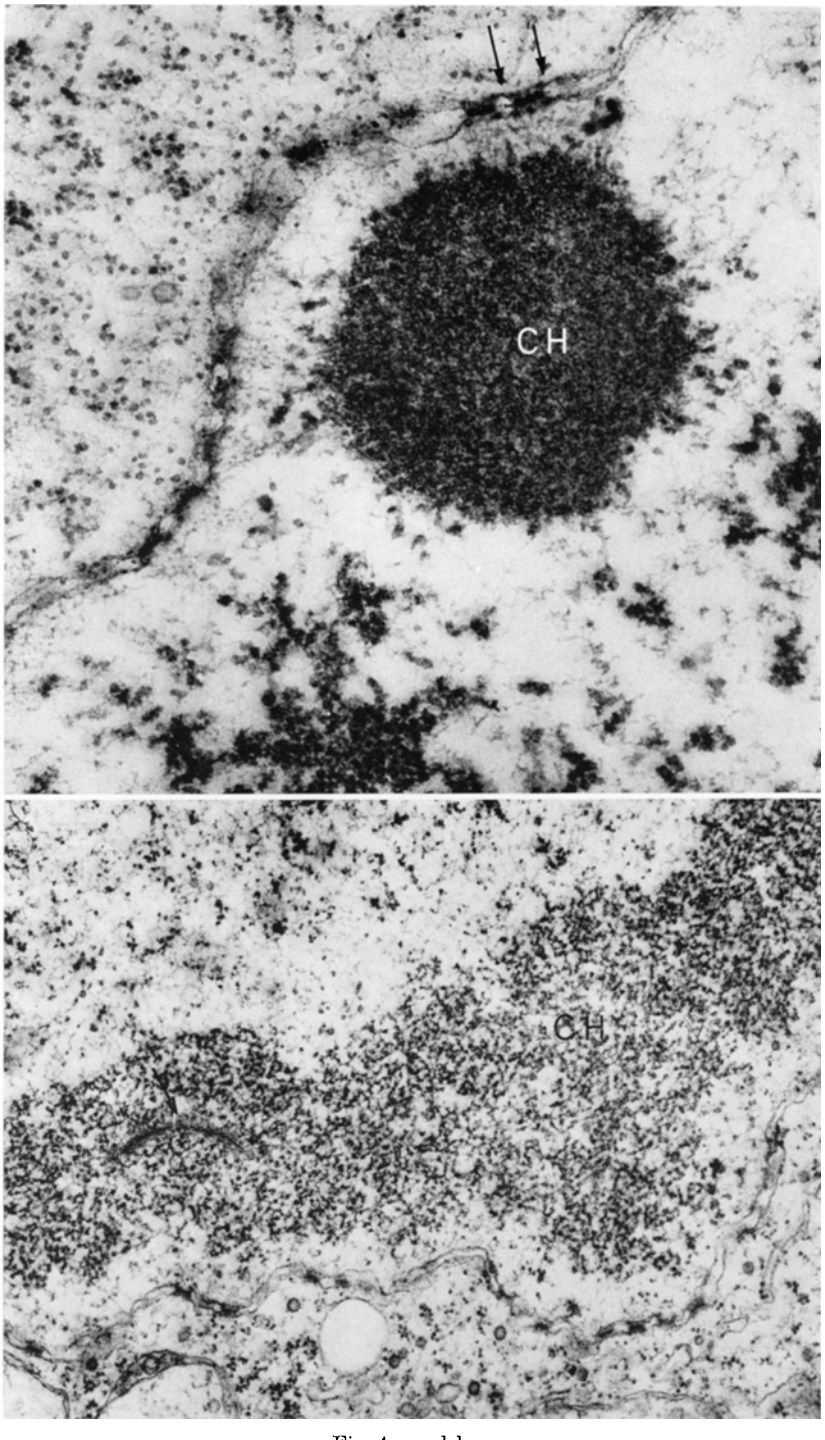
Studies on fragments of envelopes isolated from animal and plant nuclei have shown that, with the aid of the negative-stain technique (Horne, 1965) and the use of optical rotation (Markham *et al.*, 1963), the annulus encircling and overlapping the pore margin on the outer surface of the envelope is predominantly an eight-fold repeating structure (e.g. Gall, 1967; Yoo and Bayley, 1967; Franke, 1966, 1967; Franke and Scheer, 1970a).

Arrays of annuli in tangential section are illustrated in Fig. 1 and 2. In annuli seen face-on (Figs. 2c, 6), it was sometimes possible to visualise the 8 members of the repeating structure (e.g. Fig. 6). These units, seemingly ovoid in shape, encircled a conspicuous globule (or plug) which was seen in most annuli. Further, it was clear that each of the units, as well as in part the plug, was made up of loosely coiled filaments about 3 nm in diameter. These appeared to be arranged in such a way that those in the repeating units were continuous with those in the plug. Their parts situated in the latter were embedded in an amorphous background substance. The plug sometimes appeared to be compacted and occasionally became irregular in shape, particularly in tangential sections. These structural features were common to all the liliaceous plants studied. The diameter of the annuli was consistently about 115–120 nm in all material, with an opening of variable width ranging from about 35–45 nm; the diameter of the plug ranged from about 25–30 nm.

3. The Pore Complex in Profile

Recent observations in animal and plant nuclei have indicated that annular material, similarly in the form of an eight-formed repeating structure, occurs also at the other end of the pore on the nuclear side (Franke and Scheer, 1970; Franke, 1970; Roberts and Northcote, 1970). Some of the repeating members at both ends of the pore were sometimes evident in profiles of the pore complex in the present material (Fig. 7).

All of our illustrations of profiles show a more or less irregular band of material in the waist of the pore of similar opacity to the annular material at the



a

b

Fig. 4a and b

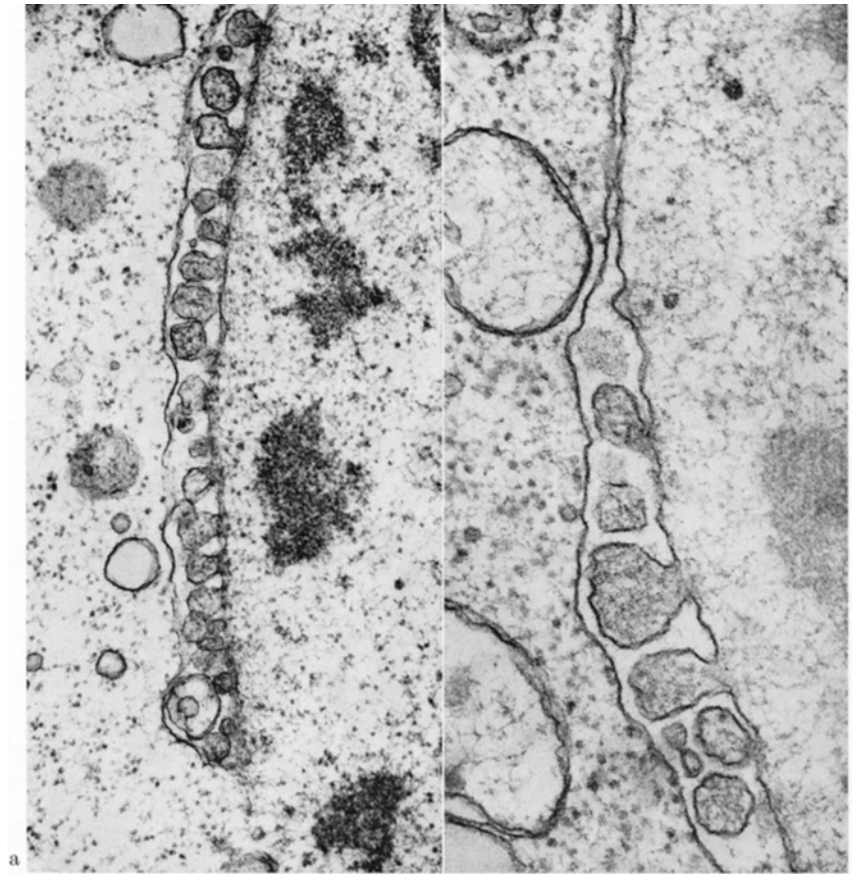


Fig. 5a and b. Micrographs of cross-linked methacrylate sections of p.m.c. of *Triticum durum* var. Aziziah, showing sacculation of inner membrane of the nuclear envelope and absence of pores. (a) mid-prophase from an asynaptic plant, ×40000; (b) leptotene from a synaptic one, × 60000

margin of the pore (Figs. 1, 4, 7). At high magnification it was clear that most of the highly opaque material within the pore was due to the presence of spherical aggregations of filaments, encircling the central plug and situated close to the wall of the pore (Fig. 7a). There is clearly a correspondence between these aggregations and the peripheral granules recently described in nuclear pores of other plant material (Roberts and Northcote, 1970). The diameter of the filaments in them was similar to those in the plug and the repeating units at the pore margin.

Fig. 4. (a) Micrograph of an Epon section of a p.m.c. at pachytene of *Fritillaria lanceolata* showing migration of material from a chromocentre (*CH*) toward the nuclear envelope; knobbed appendages (indicated by arrows) can be seen extending laterally from a pore complex into the perinuclear space at both sides, ×60000. (b) from a p.m.c. at zygotene of *Fritillaria mutica*, showing irregular distribution of pores in part of envelope adjacent to a chromocentre and less prominent movement of material toward it; an axial core is indicated by arrow, × 30000

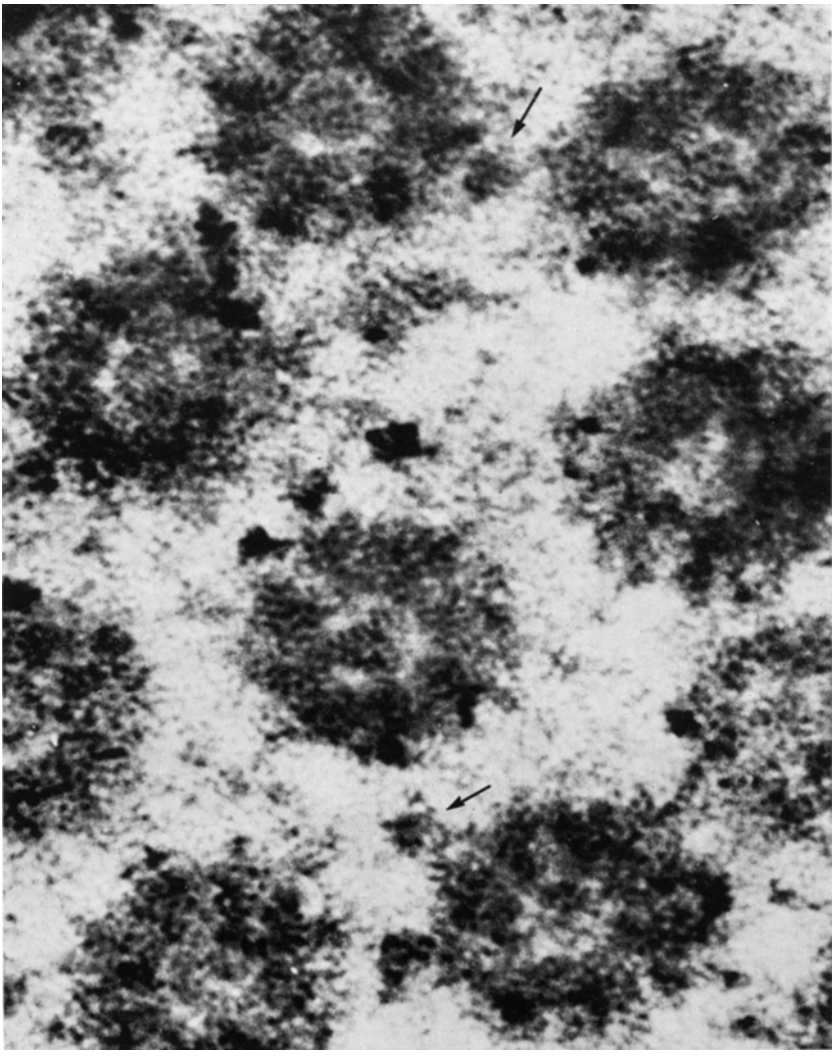


Fig. 6. Face-on view of annuli in a p.m.c. at zygotene of *Fritillaria lanceolata*, showing the fibrillar composition of the subunits and the central plug; ribosomes (indicated by arrows) can be seen on the surface of the envelope, × 400000

The remaining area within the pore always appeared to be occupied by less opaque amorphous material.

In some sections knobbed filaments were seen extending laterally from the wall of the pore into the perinuclear space at two sides, rather as if filaments from inside the pore pierce the wall (Fig. 4a).

4. Other Features

Some measurements made in profiles of the dimensions of pores in nuclei at zygotene in the lily Formobel and *F. lanceolata* indicate that they have a dia-

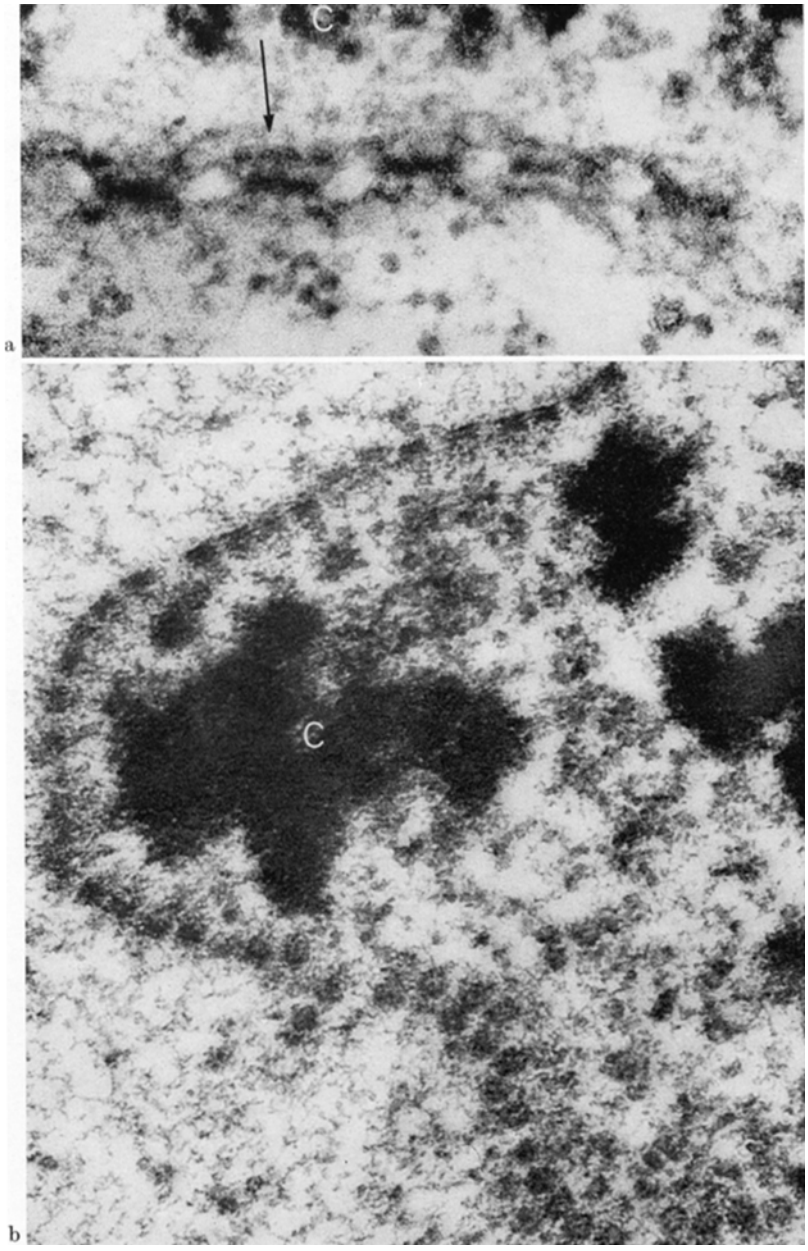


Fig. 7. (a) Micrograph of pore complexes in profile from a p.m.c. at pachytene of *Fritillaria lanceolata*. The central plug and some of the internal and external subunits of the annulus are clearly visible in the pore complex marked by arrow, × 160000. (b) Pore complexes in perpendicular and tangential sections stained with ethanolic phosphotungstic acid alone; the central plug and components of the annulus are stained but not the membranes of the envelope, × 45000

meter of about 75 nm. This may be a slight underestimate, since in thin sections an accurate determination of the pore diameter will be dependent on coincidence of the plane of section with the pore centre, assuming the pore has a circular outline.

Electron microscopical studies have shown that cell components containing basic proteins stain intensely with ethanolic phosphotungstic acid (Bloom and Aghajanian, 1969), probably by binding with lysine and arginine residues (Sheridan and Barrnett, 1969). The latter workers found that nuclear pores showed prominently in sections of p.m.c. of *L. longiflorum* stained in this way. Our observations with this staining method show that, although the membranes of the envelope do not stain, the fibrils of the annular components and central plug stain intensely (Fig. 7b). A similar staining behaviour was observed with uranyl acetate when this was used in the way suggested by Hills and Plaskitt (1968) as a protein stain.

In both *F. lanceolata* and *F. mutica* the prophase nuclei in p.m.c. show 1–4 chromocentres which persist until pachytene (La Cour and Wells, 1970). These bodies which derive from fusion of heterochromatic segments situated proximal to the centromere in most of their chromosomes, always lie close to the nuclear surface and might therefore be expected to engage in nucleocytoplasmic exchange. Fig. 4a illustrates the migration of material from a chromocentre towards the envelope in a pachytene nucleus of *F. lanceolata*, but actual passage of material through the pores from these bodies was not observed. A less obstrusive movment of material from a chromocentre in a nucleus of *F. mutica* during zygotene is shown in Fig. 4b.

Discussion

Our observations in p.m.c. of four liliaceous plants suggest that, as a general rule, in these cells pores are irregularly distributed over the nuclear envelope in clusters of variable size from leptotene to pachytene. Previous studies of the distribution of pores in plant nuclei, all of them in root tips, have also shown an irregular, though perhaps slightly dissimilar, pattern of distribution (Branton and Moor, 1964; Franke, 1966; Yoo and Bayley, 1967; Nothcote and Lewis, 1968). In somatic nuclei, judging by these authors' illustrations, they appear to be generally more widely spaced and therefore perhaps slightly more evenly distributed.

The sparseness of pores in the meiotic nuclei of the synaptic and asynaptic wheat would seem to suggest that a large number of pores is not really required by these nuclei during early to midprophase. Some support for this view comes from observations which indicate that there is most likely a low incidence in passage of material from the nucleus to cytoplasm at this time. For example, in p.m.c. of *Lilium henryi* and *Trillium erectum* Mackenzie *et al.* (1967) have shown that there is a decline in RNA in mid-prophase which is associated with a pronounced reduction in the number of ribosomes; this latter event was also noticeable in our pachytene preparations. These changes are accompanied evidently also by a decline in RNA synthesis in the nucleolus during prophase, as noted in *Zea mays* (Das, 1965) and the Chinese hamster (Utakoji, 1966).

In addition there are a number of observations indicating a variation in number of nuclear pores according to the metabolic state of the cell. Oocytes of amphibia

(Afzelius, 1955; Franke and Scheer, 1970b), the macronucleus of *Tetrahymena pyriformis* (Franke, 1967) and salivary gland nuclei of diptera (Wiener *et al.*, 1965) all have a high number of pores and intense metabolic activity. A much lower number has been found in nuclei of old yeast cells (Moor and Mühlethaler, 1963), acidophil cells in mammalian tissue (Barnes and Davis, 1959) and late erythroblasts of mammalian foetal tissue (Grasso *et al.*, 1962), all with a low metabolic activity.

Our observations have shown that pores are not present in the region of the envelope where the nucleolus is tightly pressed against it, as at leptotene in some lilies and *F. lanceolata*. The most plausible explanation for this would seem to be a physical one, since in these plants the positioning of the envelope, which must arise as a result of a pre-alignment of homologous chromosomes, was determined already at telophase of the premeiotic mitosis. That is, at the time when the envelope and pores were laid down. If it is necessary for the newly forming envelope to be in contact with the surface of the chromosomes for pores to be formed, as some observations on reconstruction of the envelope seem to suggest (Moses, 1964; Robbins and Gonatas, 1964; Murray *et al.*, 1965), the adpressed developing nucleolus may be an obstacle to their formation.

Although it can be assumed that pores are mostly formed during reconstruction of the envelope at telophase, there is indication that, at least in particular types of cell, their number can increase with increasing metabolic activity of the nucleus, as in oocytes of amphibia (Franke and Scheer, 1970b). It is not known whether pores are sometimes short-lived.

The annuli which encircle the pores at the outer surface of the nuclear envelope have been repeatedly illustrated in sections of animal and plant tissues cut tangential to the nuclear surface after various methods of fixation, except permanganate (Marinos, 1960; Merriam, 1961; Gall, 1964). A hint as to the granule-like form of the subunits involved in the eight-fold symmetry in their structure dates back to early observations in animal nuclei (Gall, 1956; Watson, 1959). On the other hand, Wischnitzer (1958) interpreted the structure as representing cross-sections of hollow tubules situated within the pore; a somewhat similar view has been advanced recently by Vivier (1967).

Our observations show that both the annular subunits at the pore margin and the central plug are composed of loosely wound filaments about 3 nm in diameter. It would seem unlikely that these have arisen as an artifact during preparative procedures (cf. Franke, 1970). Further, as seems clearly indicated by Fig. 6, these filaments appear to be continuous with those in the plug. We may note that radial symmetry not only for the subunits but also for fine filaments radiating from them toward the plug was observed by Franke (1970) in pore complexes in nuclear envelopes of *Allium cepa* roots, after rotation analysis. Yoo and Bayley (1967) also noted similarly disposed thread-like connections in negatively stained pore complexes of nuclei isolated from apical buds of peas. We found it impossible to decide whether the filaments of the subunits within the pore, which were first described by Roberts and Northcote (1970), were likewise linked to those in the plug.

In a recent model (Franke, 1970) has indicated that filaments from the subunits at both ends of the pore and also the plug protrude for some distance into the

cytoplasm and nucleoplasm, respectively. We have yet to be convinced that this occurs in our material. In profiles, threads of similar width to the filaments in the annular subunits sometimes appear to be radiating from the pores, but their opacity is somewhat lower.

In order to preserve a necessary degree of rigidity in structure, it is clear that the ends of the filaments, or some ends at least, in all subunits must be bonded to the wall of the pore. In the preparation of amphibian oocyte nuclear envelopes, the subunits and plug are not seen when divalent cations such as Mg^{2+} or Ca^{2+} are omitted from the isolation media (Franke and Scheer, 1970), possibly because of cleavage of these bonds. The knobbed appendages seen extending from the pore wall into the perinuclear space in some profiles (Fig. 4) are perhaps connected with bonding of the ends of filaments from the subunits within the pore, although the thickness of these appendages away from the knob is about twice that of the filaments. Picheral (1970), who observed similar structures on pores in the envelopes of spermatid nuclei of a newt, has interpreted them as representing external edges of a diaphragm which is independent of the annulus. The presence of a diaphragm as such is not supported by the present observations and the existence of one has long been open to question (see Watson, 1955, 1959; Merriam, 1961, 1962; Du Praw, 1965; Wiener *et al.*, 1965; Ward and Ward, 1968; Scharrer and Wurzelmann, 1969; Stevens and André, 1969).

The variously named "central-dot", "-granule", "pore plug", "globule", "plug" has been described many times in pores of animal and plant nuclei (e.g. Pollister *et al.*, 1954; Afzelius, 1955; Swift, 1958; Merriam, 1961, 1962; Vivier, 1967; Franke and Scheer, 1970a, 1970b). The frequency of pores with plugs appears to vary with different tissues (see Comes and Franke, 1970). Recent observations suggest that it is highest in envelopes of cells most actively engaged in RNA synthesis, as for example in amphibian oocytes at the lampbrush stage (Franke and Scheer, 1970b). Moreover, in the envelopes of such nuclei at the same stage, the frequency of pores with plugs is reduced when RNA synthesis is blocked by incubation of oocytes in actinomycin (Scheer, 1970). On the other hand, in the present material, where the cells were at or approaching a low ebb of RNA synthesis, most of the pores appeared to contain plugs.

Our observations agree with those of Franke and Scheer (1970a) in that the diameter of the plug is somewhat variable, but we did not see any that were composed of more than one aggregate of fibrils comparable to the smaller granules of Yoo and Bayley (1967) and Kessel (1969).

The annulus is removed specifically by pepsin treatment of thin Epon sections (Beaulaton, 1968), but the evidence that it is sensitive to RNase is open to question (Mentre, 1969; cf. Beaulaton, 1968; Du Praw, 1965; Merriam, 1961). This suggests that the filaments are mainly or wholly protein in nature. The intense staining we obtained with ethanolic PTA (Fig. 7b) suggests that, from evidence presented by Sheridan and Barrnett (1969), the protein is basic in character. Since, as our observations suggest, the filaments in the subunits are continuous with those in the plug, it is not surprising that the plug reacted similarly to the PTA. According to Mentre (1969), the plug is more sensitive to RNase digestion than the annulus.

A model of the pore complex based on the present observations is shown in Fig. 8. Of three other models which have been proposed recently, it follows more

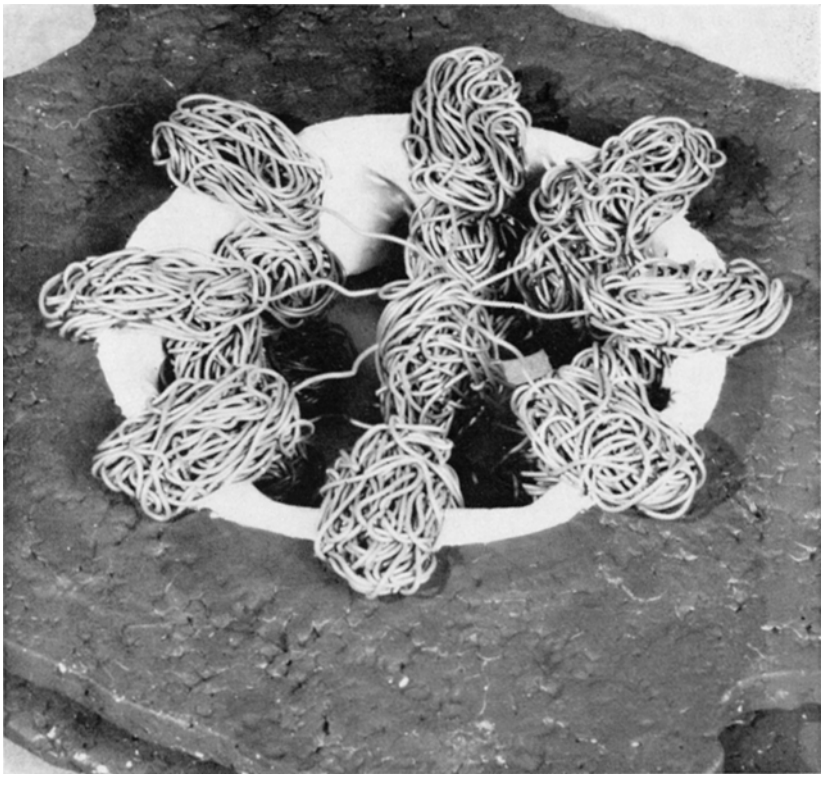


Fig. 8. A model of the pore complex based on the present observations and those of Roberts and Northcote (1970). It is symmetrical on both the cytoplasmic and nuclear sides, respectively. Amorphous material observed in the central plug and that which fills the pore is not shown. The lip seen at the pore margin is seen only in negative-stain preparations and is most probably part of the pore wall. We consider it unlikely that filaments run from the plug to the spherical subunits of the annulus situated within the pore

closely that of Roberts and Northcote (1970), but like this model it differs only slightly from the "fibrillar" one of Franke (1970). This is in respect of the subunits of the annulus within the pore and the shape of the subunits at the pore margin. All of these models are at variance with the one proposed by Abelson and Smith (1970) which involves the inclusion of central tubules and minitubules within the pore.

A conspicuous feature of the pore complex is the apparent universality of its intricate fine structure. Although it is obvious that the pores must provide a pathway for nucleocytoplasmic transfer of macromolecules, the means by which this is controlled has remained obscure. All the available evidence shows that egression of material is confined to a pathway through the pore centre with a limit in cross dimensions of about 15 nm (e.g. Anderson and Beams, 1956; Feldherr, 1962, 1965, 1969; Feldherr and Harding, 1964; Beermann, 1964; Stevens and Swift, 1966; Franke and Scheer, 1970b). No clearly defined channel is apparent

through the pore centre and obviously there must be a barrier to free diffusion. As already suggested by Roberts and Northcote (1970), the amorphous material within the pore probably provides this barrier. The units of the annulus situated within the pore (Figs. 7 and 8) will make some contribution to limiting the size of the exit.

By reason of its position and complex structure there is some justification for considering the possibility that the annulus is in some way involved in opening and closing the pore. Such a role has been assigned by Kessel (1969) to the central plug. If this is indeed so, it is tempting to consider that the filaments in the annulus may have contractile properties. Our observations suggest that they are continuous with those in the plug and this could provide a means of moving it up and down, or to one side. On the other hand, the variability in size of the plug would not seem to favour its utilization as an actual plug. Stevens and André (1969) have suggested that the annulus is probably endowed with enzymatic activities which determine when pores are penetrable and impenetrable.

There is a growing inclination to regard the plug as a transitory structure representing ribonucleoprotein in transit through the pores (Stevens and André, 1969). Some support for this view comes from Scheer's (1970) observation that incubation of amphibian oocytes in actinomycin leads to a reduction in the number of pores containing the plug. Mepham and Lane (1969) have suggested that nuclear pores are sites at which polyribosomes are assembled before leaving the nucleus. Although it is not unlikely that ribosomes may be assembled at the pores prior to transit from the nucleus, we doubt very much that ribosomal aggregates are in some way derived from the annular components as they suggest. Some alternative relationships involving nucleocytoplasmic ribonucleoprotein transport to these components have been noted by Franke and Scheer (1970b). It is conceivable that annuli are involved both in the assembly of such macromolecules and in regulation of their transit through the pore.

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