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ORIGIN AND DEVELOPMENT OF KINETOSOMES IN *OXYTRICHA FALLAX*

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SUMMARY

Two significant features of the origin and development of kinetosomes in the ciliate, *Oxytricha fallax*, are indicated by electron micrography of serial sections. (1) Some kinetosomes arise in varied orientations to adjacent kinetosomes instead of in the single orientation regularly found in other material; and some kinetosomes arise in positions that are not adjacent to any kinetosome. Hence, even in ciliates, some kinetosomes arise without using existing kinetosomes or their micromilieu as nucleation sites. (2) Growth of kinetosomal microtubules appears to occur at their proximal as well as distal ends. This is indicated by proximal cross-sections with only one or two microtubules of a triplet, while more distal sections of the same incompletely grown kinetosome show the complete triplet pattern.

INTRODUCTION

Kinetosomes (ciliary basal bodies) and centrioles are known to arise in many organisms perpendicular and adjacent to the proximal wall of a mature kinetosome or centriole, respectively (e.g. Allen, 1969; Dippell, 1968; Millechia & Rudzinska, 1970; Sonneborn, 1970); but other orientations and positions are also known (e.g. Anderson & Brenner, 1971; Dirksen & Crocker, 1966; Gall, 1961; Johnson & Porter, 1968; Kalnins & Porter, 1969; Sorokin, 1968; Steinman, 1968). Even origin in the complete absence of the mature organelle has been reported (Dingle & Fulton, 1966; Fulton & Dingle, 1971) in *Naegleria*. Many questions remain unanswered regarding origin and development of kinetosomes and centrioles. For this reason, certain favourable features of the morphogenetic processes of the ciliated protozoon, *Oxytricha fallax*, were exploited in order to investigate certain aspects of kinetosomal origin and development.

A large field of rapidly proliferating kinetosomes (the oral primordium) is present in *O. fallax* in a definite location on the cell surface at an identifiable stage of the cell cycle, about midway through the macronuclear *S* period (recognized as set forth below). During later stages of development, successive rounds of kinetosome production occur at a precise location, on the anterior right margin, then proceeding left, of each developing membranelle (Grimes, 1972), thus making it possible to recognize the relative ages of the kinetosomes.

Using this material, kinetosomes have been shown, for the first time in ciliates, to arise in varied orientations to adjacent mature kinetosomes, and sometimes not adjacent to any mature kinetosome. In addition, observations are presented on kinetosome development, including material which indicates unequal microtubular elongation at the proximal ends of developing kinetosomes.

MATERIALS AND METHODS

The strain of *Oxytricha fallax* used in this study was isolated by Jack Swelstad from a small stream on the Bloomington campus of Indiana University. The cultural and electron-microscopic techniques used were described previously (Grimes, 1972).

Flat-embedded cells in the desired stage of the cell cycle were identified by observing the location in their macronuclei of a characteristic transverse band (the reorganization band) which passes from one end of the macronucleus to the other during the *S* period in precise correlation with the pre-fission sequence of cortical events. For example, when the band is about midway through macronuclei, the oral primordium is present and its kinetosomes are rapidly increasing in number. In like manner, cells in any desired stage of pre-fission morphogenesis can be selected for sectioning by picking cells showing the correlated position of the macronuclear reorganization band.

Nascent kinetosomes are those kinetosomes in the process of establishing the triplet pattern. These are distinguishable from juvenile kinetosomes, i.e. kinetosomes in their growth phase, subsequent to formation of the triplet pattern. Serial sections show that a kinetosome confined to a single section is nascent. Juvenile kinetosomes are always found in more than one section of a set of serial sections.

RESULTS

Orientation of newly forming kinetosomes

New kinetosomes sometimes arise in the 'typical' orientation and position, i.e. perpendicular and adjacent to the proximal wall of an existing kinetosome, in such a way that their longitudinal axes intersect, or nearly intersect, as shown in Figs. 11–13; but other orientations also occur. One such orientation, illustrated by Figs. 4–6, is for the longitudinal axis of the new kinetosome to be parallel instead of perpendicular to those of adjacent mature kinetosomes, and hence perpendicular instead of parallel to the cell surface. Because this orientation is characteristic of mature kinetosomes, the validity of the preceding description depends upon the validity of the identification of the kinetosomes as nascent. The criterion of confinement to a single section of a series (see Methods) is met by the kinetosomes in Figs. 4–7. As appears in the 3 serial sections of Fig. 4A–C, the nascent kinetosome appears only in the middle section (4B). The other criterion (incomplete structure of the microtubular wall, i.e. fewer than 3 microtubules per fibre) is also met in Figs. 4–7.

Developing kinetosomes show other orientations. Some are oriented with their longitudinal axes neither parallel to nor intersecting the longitudinal axes of the adjacent kinetosomes (Figs. 3, 8). Within this category the angle of divergence from perpendicular is variable ($\sim$ 90° in Fig. 3, $\sim$ 120° in Fig. 8). Some are not adjacent to an existing kinetosome (e.g. the kinetosome at the arrow in Fig. 2).

Certain other structures, such as membranes (Figs. 2–4) and the fibrous material of the cirral basket (Fig. 8), are frequently close to atypically oriented kinetosomes. However, the significance of these associations is unclear. These structures are so common that the associations may be random. The observations are consistent with the 'organizer hypothesis' recently proposed by Anderson & Brenner (1971). However, it is difficult to imagine any orientations or localizations that would be inconsistent with this model; hence the usefulness of the model depends upon the still unachieved demonstration of the organizer substance.

Formation of the triplet pattern

The nascent kinetosomes in Figs. 4–7 represent stages in the formation of the triplet pattern. A full series of developmental stages has not been obtained, but the observed stages indicate a sequence which is, at least in general, consistent with the sequence reported by Dippell (1968) for the kinetosomes of *Paramecium*, including the presence of linkers (Fig. 7).

Kinetosome growth after formation of the triplet pattern

Unequal growth of the microtubules of juvenile kinetosomes at the distal end has been stated by Anderson & Brenner (1971) to occur in the Rhesus monkey. Figs. 4A–C and 9–13 present observations which are interpreted to indicate the same phenomenon for the proximal (cartwheel) end of juvenile kinetosomes in *O. fallax*. That these are juvenile kinetosomes is evident from serial sections; for example, some kinetosomes in Figs. 4A (double arrow), 9A, B, and 10A (at arrows) show the complete triplet pattern. The next proximal sections (4B, 9B, C and 10B, respectively) show that the microtubule walls of the indicated juvenile kinetosomes are incomplete (less than 3 microtubules per fibre), and vary as to the extent of completeness. Such images are commonly observed among juvenile kinetosomes. These figures also illustrate the unique hub morphology which is common to all immature kinetosomes (compare with hub of mature kinetosomes in Fig. 1).

Arranging the juvenile kinetosomes described above according to the number of microtubules at their proximal ends yields a series that could indicate the sequence of microtubular growth at the proximal end of maturing kinetosomes. The indicated sequence is for the A tubule to elongate proximally first, then the B tubule, and finally the C tubule. If this interpretation is correct, then growth at the proximal end appears similar to growth at the distal end as reported by Anderson & Brenner (1971).

Further support of unequal proximal tubule elongation in maturing kinetosomes is provided by observations on longitudinal sections of developing kinetosomes. Figs. 11–13 illustrate the following major points: (1) The outer tubules of the kinetosomal wall are shorter than the inner tubules at the proximal end (as described above) and probably also at the distal end (as reported by Anderson & Brenner, 1971), thus indicating tubule growth at both ends of the kinetosomes. (2) The central hub of the developing kinetosomes extends below the proximal margin of the kinetosomal wall (as it does to a lesser extent in the mature kinetosome, Fig. 1). (3) The hub elongates as the kinetosome grows, until the hub length is comparable to that found in mature kinetosomes ($\sim$ 100 nm). The hub must then be growing at the distal end, or growing at the proximal end and sliding into the kinetosome, or growing at the proximal end with the tubules of the kinetosomal wall also growing proximally.

DISCUSSION

Material has been presented which implies 2 important aspects of kinetosome development in *O. fallax*. The first of these is that tubule walls of juvenile kinetosomes

grow at the proximal as well as at the distal end. However, the described images could be explained in other ways. Unequal lengths of microtubules within a triplet could result from resorption or slippage of microtubules. However, either of these possibilities would make the formation of new kinetosomes less efficient, because microtubules within triplets share common walls. In addition, growth at the proximal end would be implied if the proximal end of the hub grows (as is indicated) and no slippage of the hub into the kinetosome occurs.

Unequal lengths of microtubules within triplets of juvenile kinetosomes also results in the production of images which closely mimic stages in the formation of the triplet pattern. Clear distinction between nascent and immature kinetosomes has been possible here only by serial sectioning. If unequal tubule length within triplets during kinetosome maturation is common among species, images previously described as nascent kinetosomes may actually represent incorrectly interpreted juvenile kinetosomes.

The second and perhaps more important feature of kinetosome development indicated is that kinetosomes frequently arise in positions and orientations other than the usual one (perpendicular and adjacent to the proximal wall of an adjacent kinetosome). It can be argued that all of the orientations described are the result of early movement after origin in the usual position and orientation. No direct evidence is available to refute this argument, but such movement, if it occurs, must occur in the earliest developmental stages. Nor is there any direct evidence of very early movement in any organism. Hence, a more straightforward interpretation is that initiation of development can occur in atypical orientations to existing kinetosomes. Similar observations with the same interpretation have been made elsewhere (e.g. Anderson & Brenner, 1971; Dirksen & Crocker, 1966; Kalnins & Porter, 1969; Martinez-Martinez & Daems, 1968; Perkins, 1970; Sorokin, 1968; Steinman, 1968) using various organisms. In general, atypical orientations are reported to occur when large numbers of kinetosomes are synthesized in regions initially possessing few kinetosomes, and/or when precise orientation at the time of kinetosome production is not essential. In *O. fallax* atypical orientations are observed in the oral primordium where the number of mature kinetosomes is initially small, but increases rapidly. Moreover, precise orientation of the kinetosomes at the time of their production is not critical; the kinetosomes do not assume their final characteristic positions until after most of the kinetosomes have been produced.

The utilization of an existing kinetosome or its immediate micromilieu as an initiation site for kinetosomal production thus appears not to be the only option available to organisms, even to organisms that use that site for production of some of their kinetosomes.

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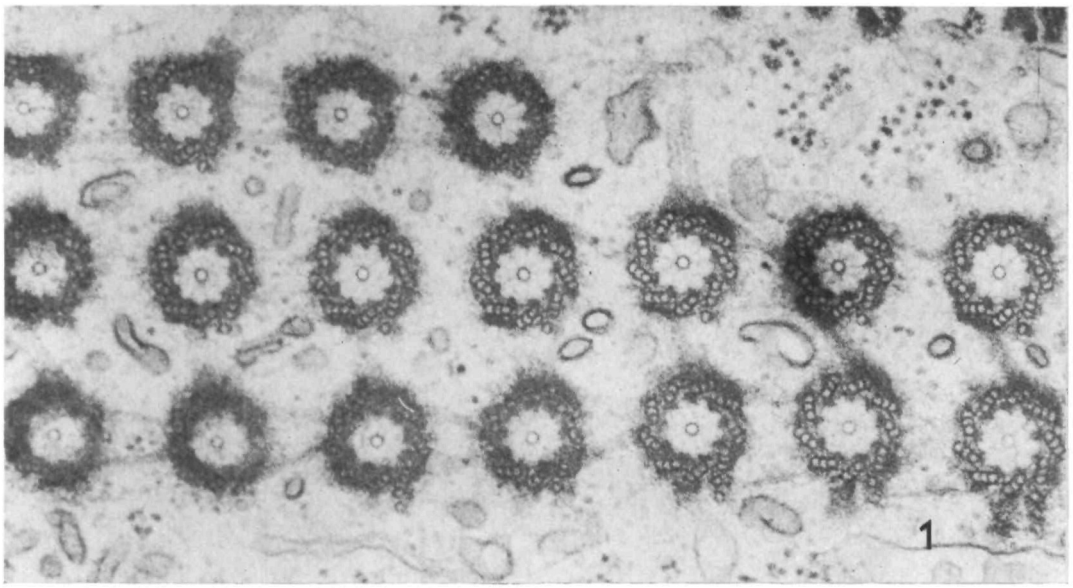
Fig. 1. Section through the proximal ends of kinetosomes in mature adoral zone of membranelles. × 75 000.

Fig. 2. A developing kinetosome (at arrow, cut through wall) illustrating development far from existing kinetosomes. Serial sections showed no kinetosomes closer than those in this micrograph. *ci*, cilium of anal cirrus no. 6. × 75 000.

Fig. 3. Section through a developing kinetosome in an oral primordium. The faint hub (at arrow) extends proximally past the margin of the microtubular wall. × 100 000.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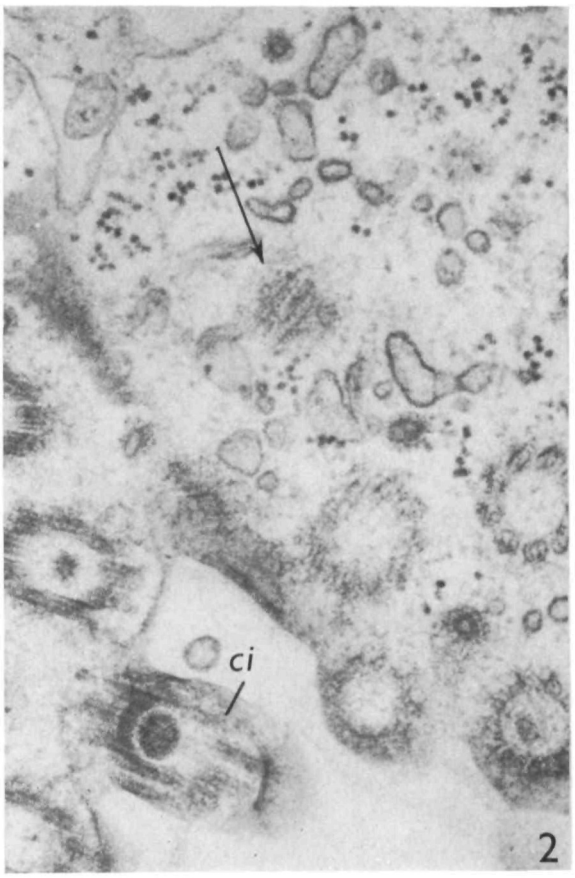
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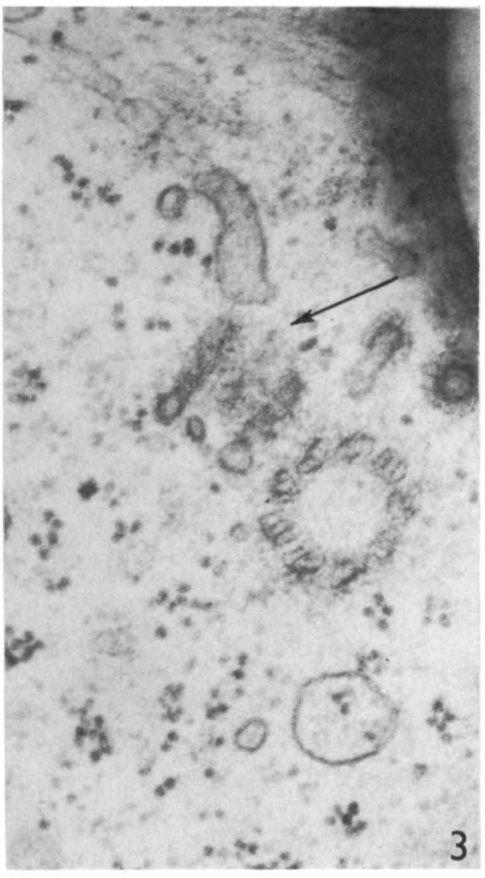
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Fig. 4. Serial sections through part of an early oral primordium cut parallel to the cell surface. *jk*, juvenile kinetosome; *nk*, nascent kinetosome. × 60000.

Figs. 5–7. Nascent kinetosomes in 3 different oral primordia: 5 and 6 are cut parallel to the cell surface, 7 perpendicular. *l*, linkers; *nk*, nascent kinetosome. × 120000.

Fig. 8. Two consecutive members of a larger set of serial sections through an early oral primordium. Arrow points to hub in A. Kinetosomes (*k*) of anal cirrus no. 6 (to the reader's right of the dashed lines), next to which the oral primordium arises. *f*, fibrous material of the cirral basket. × 75000.

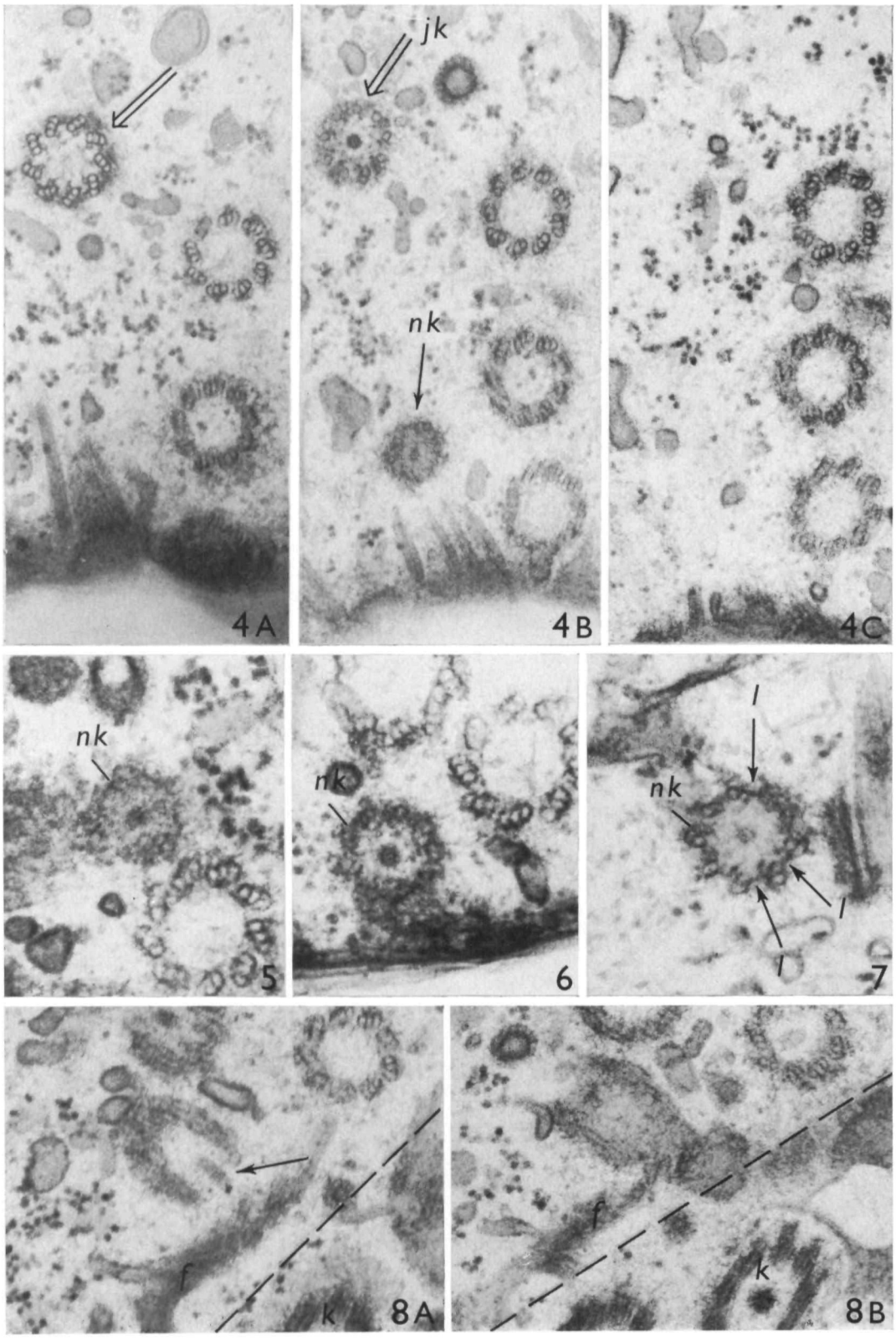
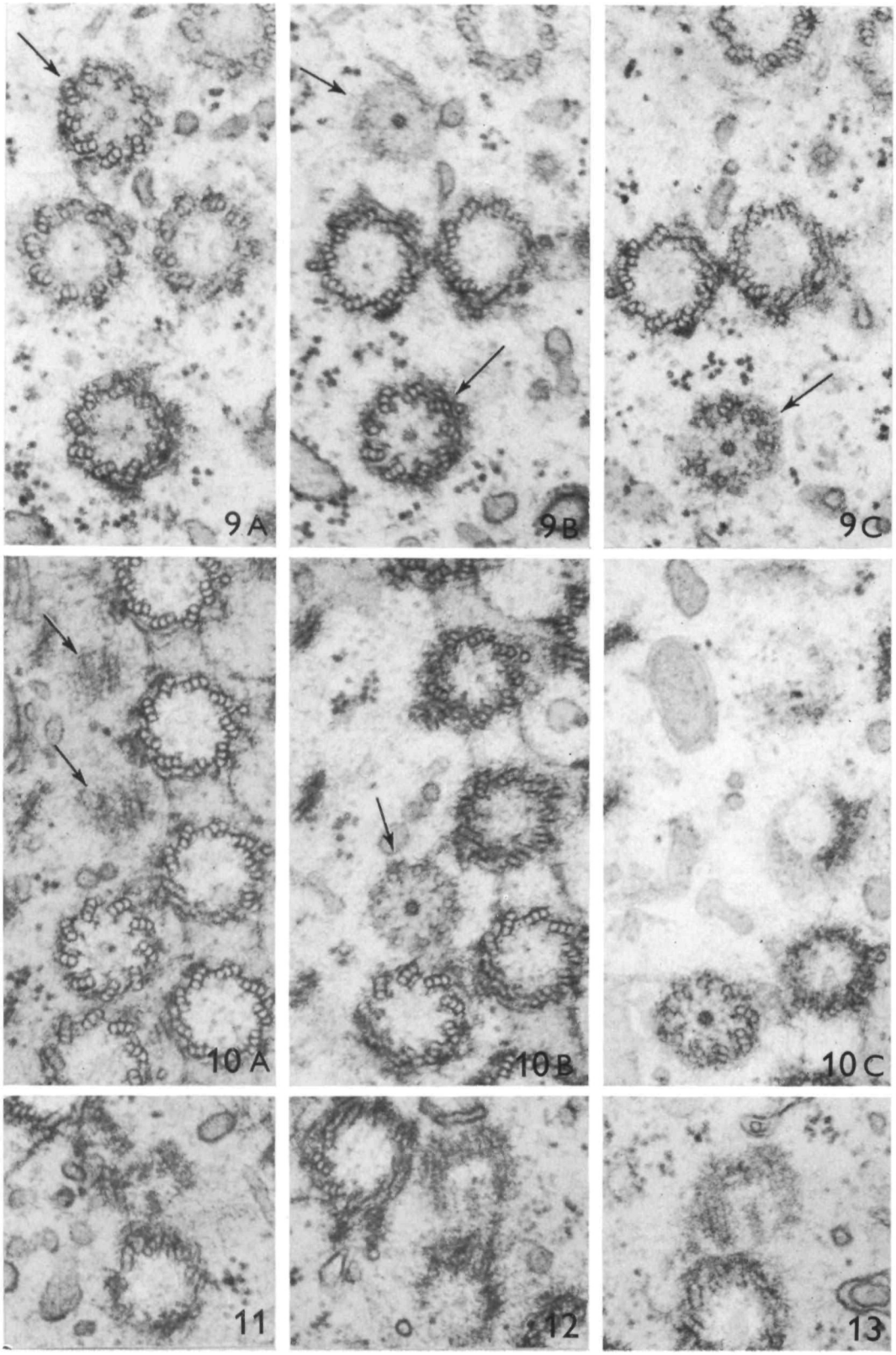

jk
nk
4A
4B
4C
nk
nk
l
nk
l
l
5
6
7
f
k
f
k
8A
8B

Fig. 9. Consecutive sections through mature and juvenile (arrows) kinetosomes in an early oral primordium. ×75000.

Fig. 10. Consecutive sections in an aligning oral primordium (left is the animal's anterior, and up is the animal's left). The proximal end of a juvenile kinetosome is in B (arrow). Arrows in A point to new kinetosomes in the forming membranelle. ×75000.

Figs. 11–13. Longitudinal sections through developing kinetosomes in 3 different oral primordia. ×75000.


9A
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