AN ORDERED FILAMENTOUS COMPONENT IN *SCIARA* (DIPTERA) SALIVARY GLAND CELL NUCLEI

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INTRODUCTION

The only structured components generally found in nuclei are chromatin (or chromosomes) and nucleoli, although membrane-bounded inclusions (5, 9), membranous structures, (3, 5, 9), annulate lamellae (4) and particulates (1, 10) have also been described. This note deals with highly structured filamentous sheets which occur in the nucleus of *Sciara* salivary gland cells. To our knowledge this component has not been previously described.

MATERIALS AND METHODS

Female fourth instar larvae and prepupae of *Sciara coprophila* were staged by counting eye spots as has been described by Garcia (2). Salivary glands were fixed in either 1 per cent OsO_4 (pH 7.6) or 6 per cent glutaraldehyde (pH 6.8) followed by 1 per cent OsO_4 (pH 7.6) (8). Fixing solutions were buffered with 0.5 M phosphate buffer. Fixation was carried out at 0 to 4°C., and glands were dissected out in cold fixative. Tissues were dehydrated in cold acetone or alcohol and imbedded in Epon 812. Thin sections were stained in 3 per cent uranyl acetate (6 to 12 hours) followed by lead citrate (7) and examined with an RCA EMU 3C electron microscope.

RESULTS

In the course of this study we have examined the salivary glands from approximately 70 animals of which about half were old fourth instar larvae (stage e) and prepupae, and half consisted of younger fourth instar larvae (Garcia's stages a to c). Filamentous sheets occur in the salivary gland nuclei of stage e larvae and prepupae with both glutaraldehyde-osmium tetroxide and osmium tetroxide fixation. The structures are most prevalent in prepupae where they occur in almost every nucleus. We have never observed filamentous sheets in fourth instar larvae younger than stage e. These filamentous sheets are present both free in the nucleoplasm (Fig. 1) and in close association with polytene chromosomes (Figs. 2, 3, and 4). Sometimes they partially (or possibly completely) surround areas of condensed chromatin (Fig. 3) and other times they are partially (or possibly completely) enclosed within dense chromatin areas (Fig. 4).

It can be seen in Fig. 5 that the filaments are very regularly arranged parallel to one another in a single plane. Individual filaments are each about 100 A in diameter and have a center-to-center spacing of 200 A. Filaments appear as dense dots in cross-section and dense lines in lateral section. When one takes into account the section thickness (500 to 700 A) it is apparent that 100 A filaments must be orientated almost completely perpendicular to the plane of section to appear as dots. This explains why the filaments usually appear as lines and why areas of lines and dots are sometimes interspersed (see Fig. 6). Sheets of parallel filaments are bent into various irregular shapes. An area of amorphous fibrous material surrounds these sheets. This material extends 200 to 300 A on either side of the filaments and is also present between the filaments.

DISCUSSION

In an investigation of the cytoplasm of *Sciara* salivary glands (6) we have failed to find any cytoplasmic component similar to the filaments described above. Therefore, it is likely that intranuclear filaments are not transferred to the cytoplasm at least in this form.

As stated above, the filamentous sheets appear at a specific stage of development (stage e) and become more prevalent as development proceeds. Garcia (2) has found that DNA puffs first occur at the same stage and increase in size as larvae grow older. It is possible, therefore, that filamentous structures are somehow related to DNA puffs although they are not directly associated with DNA puff loci (Fig. 1).

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FIGURE 1 A portion of the nucleus of a giant salivary gland cell showing polytene chromosomes. Regions of compacted chromatin (bands) (*C*), a DNA puff (*P*), and nucleoli (*N*) can be seen in the nucleus. A small portion of cytoplasm appears in the lower right. Filamentous sheets occur in nucleoplasm (arrows). One of these filamentous structures is enlarged in the insert. Glutaraldehyde-OsO_4. × 8000; insert, × 36,000.

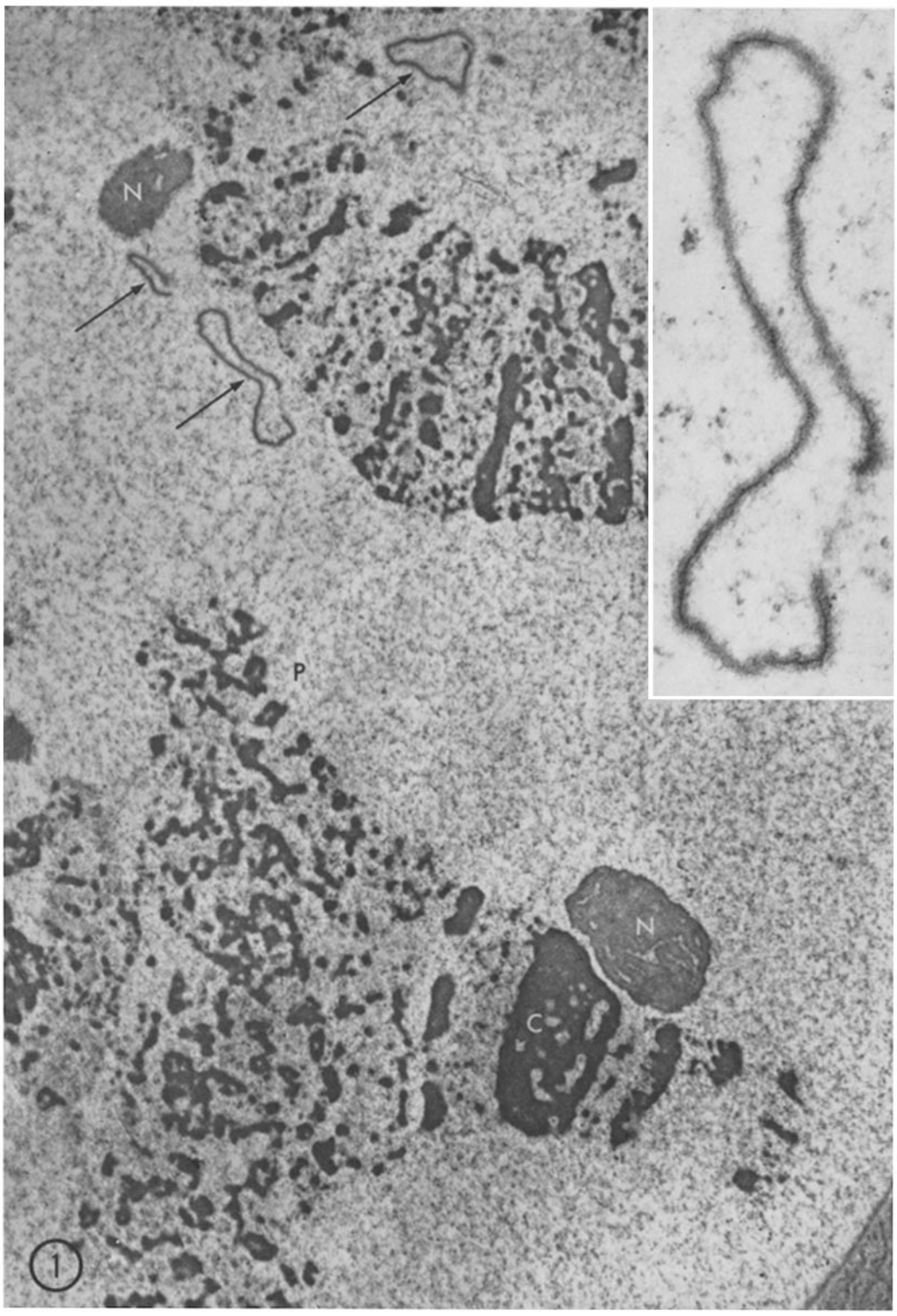

FIGURE 2 A portion of a polytene chromosome with the chromosomes running from top to bottom of the figure. Filamentous sheets (arrows) occur inside the limits of the chromosome in close association with a band region (*C*). A portion of a nucleolus can be seen in the upper right (*N*). Glutaraldehyde-OsO_4. × 25,000.

FIGURE 3 Filamentous sheets in close association with areas of chromatin. One of the filamentous sheets (arrow) partially surrounds an area of dense chromatin. Glutaraldehyde-OsO_4. × 26,000.

FIGURE 4 Portion of a polytene chromosome with a filamentous sheet (arrow) inside a chromosome band. Glutaraldehyde-OsO_4. × 17,000.

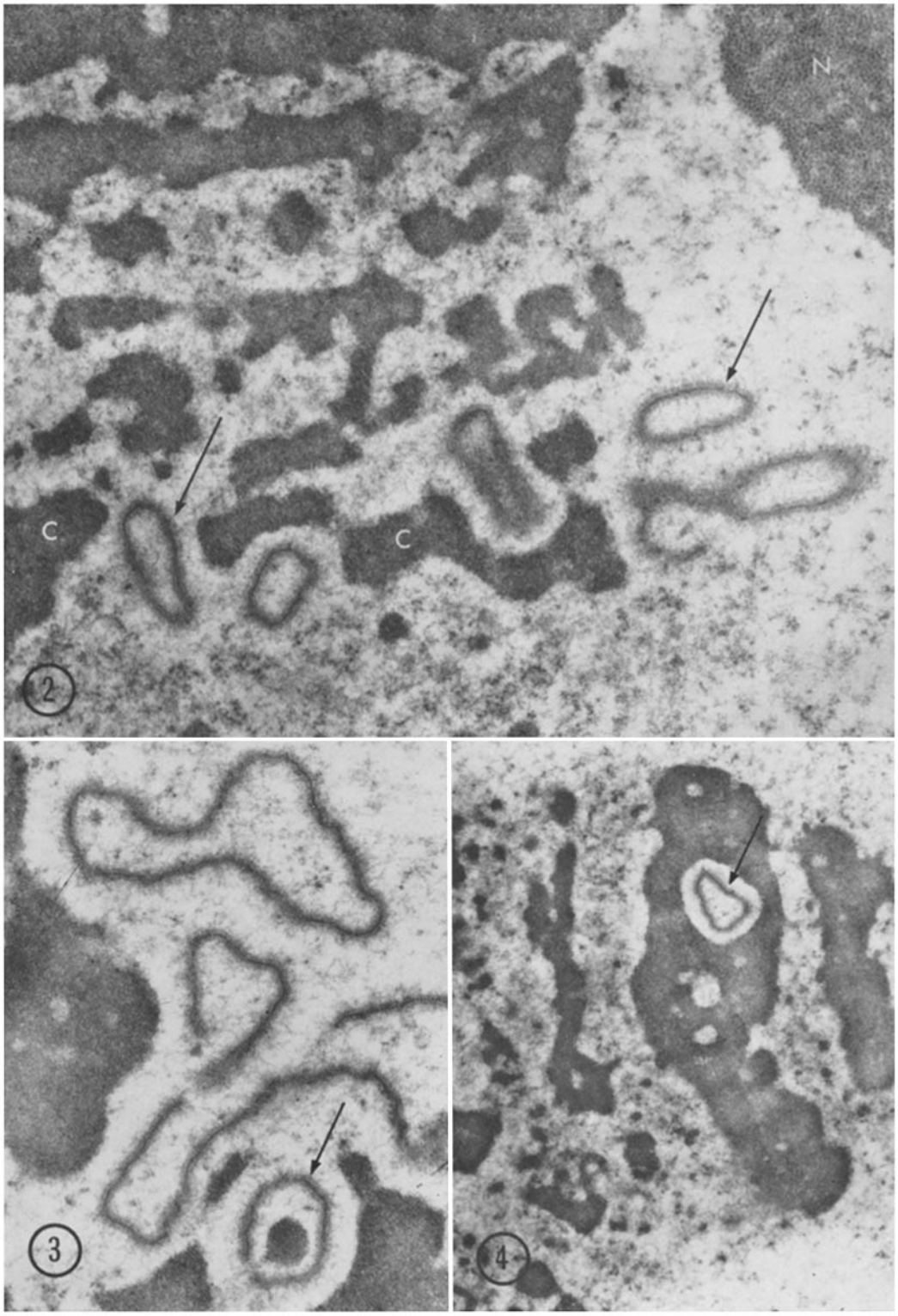

FIGURE 6 Diagram illustrating why 100 A filaments sometimes appear as interspersed regions of lines and dots when viewed in 500 to 700 A sections.

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FIGURE 5 Filamentous sheets cut in transverse and longitudinal section. Sheets are composed of regularly arranged 100 A fibers with a center-to-center spacing of 200 A. Dense amorphous fibrous material occurs around and between the filaments. × 61,000.