DISTRIBUTION OF MICROTUBULES IN THE GOLGI APPARATUS OF EUGLENA GRACILIS

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SUMMARY

A system of microtubules has been demonstrated as a consistent feature within the Golgi apparatus of Euglena gracilis. The microtubules are between 12 and 17 nm in diameter and are usually positioned in the intercisternal space between adjacent cisternae. There are seldom more than 2–5 microtubules per dictyosome section, and these may be distributed variously throughout the stack. At least some of the microtubules are continuous with the surrounding cytoplasm, so they may act to transfer products into or out of the Golgi apparatus or as structural elements to orient or position the dictyosomes and secretion vesicles.

INTRODUCTION

That extra-cisternal substances exist within the Golgi apparatus has been recognized for some time (Mollenhauer, 1965; Mollenhauer & Morré, 1966; Morré, Mollenhauer & Bracker, 1971; Sjöstrand & Hanzon, 1954), even though they cannot always be demonstrated by the usual methods of specimen preparation. These substances occupy at least 20–40% of the Golgi apparatus volume and take the form of zones of exclusion (Mollenhauer & Morré, 1972, 1973; Morré et al. 1971), intercisternal elements (Cunningham, Morré & Mollenhauer, 1966; Mollenhauer, 1965; Mollenhauer & Morré, 1966, 1972, 1973; Turner & Whaley, 1965), bonding substances (Mollenhauer & Morré, 1966; Mollenhauer, Totten & Acuff, 1971; Mollenhauer & Morré, 1972, 1973), and other elements or structures which are occasionally reported (Amos & Grimstone, 1968; Kartenbeck & Franke, 1971).

That these various constituents exist cannot be doubted, but what each does functionally is still a matter for speculation. Since they surround the Golgi apparatus (Mollenhauer & Morré, 1972, 1973; Morré et al. 1971), hold dictyosomes together (Mollenhauer & Morré, 1966; Mollenhauer et al. 1971; Mollenhauer & Morré, 1972, 1973), and alter or position dictyosome structure (Mollenhauer, 1965; Mollenhauer & Morré, 1972), they must play a significant role in Golgi apparatus function. The principal deterrent to understanding the intercisternal region is that its components cannot be seen in most electron-microscopical preparations and cannot be selectively extracted, nor recognized when isolated. Moreover, constituents like intercisternal fibres (Mollenhauer, 1965; Turner & Whaley, 1965) and bonding plaques (Mollenhauer & Morré, 1973), have so far been demonstrated only in a few plant cells and do
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not seem to be present in animal cells. Yet, the ubiquity of known Golgi apparatus structure suggests that at least somewhat comparable structural elements should exist in all Golgi apparatus, whether plant or animal, and should eventually be recognized and functionally characterized.

This study reports the presence of tubules within the intercisternal region of *Euglena* Golgi apparatus which are similar to structures commonly designated as microtubules (Hepler & Fosket, 1971; Lane & Treherne, 1970; Ledbetter & Porter, 1963; Leedale, Leadbeater & Massalski, 1970; Newcomb, 1969; O'Brien, 1967; Porter, 1966; Schnepf & Deichgrüber, 1972; Shay, 1972; Steer & Newcomb, 1969; Tilney, 1971; Tucker, 1972; Witkus, Grillo & Smith, 1969; Yamada, Spooner & Wessells, 1971). These Golgi apparatus microtubules exist at all levels within the stacks of cisternae and may be at various angles to one another. Many are continuous with the surrounding cytoplasm and could act as transport, as well as structural, elements of the Golgi apparatus.

**MATERIALS AND METHODS**

The cells, *Euglena gracilis* (Klebs) 'Z' strain, were grown axenically at 24 °C in a modified Hunter medium (Mollenhauer, Evans & Kogut, 1969). Cultures were grown in cotton-stoppered 500-ml Erlenmeyer flasks containing 100 ml of media, in the dark, and without shaking. They were exposed to room illumination for about 1-2 h prior to being fixed for microscopy.

Cells were prefixed in 0.05 M sec-collidine-buffered glutaraldehyde (2 %)-paraformaldehyde (2 %) for 45 min, rinsed well in buffer for 1—2 h and postfixed in sec-collidine-buffered OsO₄ (1 %) for 2 h. The cells were then rinsed in several changes of distilled water and post-stained in 0.5 % uranyl acetate for about 18 h. All steps were carried out in the cold (about 1-2 °C), except for the first 15 min of the aldehyde fixation, which was at room temperature. The cells were dehydrated in an ethanol-acetone series (at room temperature) and embedded in an Araldite-Epon mixture as described previously (Mollenhauer, 1964). Sections were post-stained for 1-3 min each in 2 % uranyl acetate followed by Reynolds lead citrate (Reynolds, 1963) prior to being examined with a Philips EM-300 electron microscope.

**RESULTS AND DISCUSSION**

Figs. 1-7 illustrate the general configuration of microtubules found in *Euglena* dictyosomes. In most instances microtubules appear within the intercisternal space (Figs. 1-4), though occasionally they may be present also within the lumina of the cisternae (Figs. 5-7). Microtubules appear to be a consistent feature of these Golgi apparatus and were visible in almost all suitably oriented dictyosomes examined. However, they did not appear in large numbers and, in most instances, averaged no more than 1-2 microtubules per dictyosome section, with a maximum of about 8-10 in opportune sections.

The distribution of Golgi apparatus microtubules is apparently nearly random, at least to the extent that they may be present at any level within the dictyosome (Figs. 1-4). The tubules may be oriented at various angles to one another (Figs. 1, 6) with a slight preferential association with the edges of the cisternae and toward the mature pole of the dictyosome. There are many instances in which several microtubules may be grouped together in a side-by-side orientation or in which one or more may be appressed to a cisternal membrane as illustrated in Fig. 1. Golgi apparatus micro-
Golgi apparatus microtubules appear to be continuous with the surrounding cytoplasm (Figs. 2-4) and so could act as elements for transferring products into or out of the Golgi apparatus, or as a means of positioning the dictyosomes.

The size of the Golgi apparatus microtubules is variable but lies within the range of 12.0–17.0 nm, with wall thicknesses of about 2.2–3.0 nm. They are much smaller than either the microtubules near the pellicle, which are in the neighbourhood of 20.5–25.0 nm in diameter, or those around the reservoir, which are about 17.0–19.0 nm in diameter (Arnott & Smith, 1969; Newcomb, 1969; author’s personal observation). Moreover, the surfaces of the Golgi apparatus microtubules seem smoother than those of the pellicle and reservoir microtubules.

Microtubules which appear equivalent to the intercisternal microtubules of the Golgi apparatus, are found also in the contractile and accessory vesicles (Fig. 5), the endoplasmic reticulum (Fig. 8), and occasionally within the peripheral vesicles and cisternae of the Golgi apparatus (Figs. 5, 7). In size and intracellular distribution they are somewhat similar to the Flimmer or mastigoneme microtubules reported for other organisms (Bouck, 1969; Heath, Greenwood & Griffiths, 1970; Leedale et al. 1970), though they do not appear to have the tapered ends characteristic of mastigoneme microtubules nor are mastigoneme microtubules characteristic of *Euglena* flagella. Nonetheless, the possibility of microtubule secretion via Golgi apparatus must at least be considered in subsequent studies of Golgi apparatus microtubules.

No experimental evidence is yet available to determine whether the Golgi apparatus microtubules are equivalent to other classes of microtubules (Behnke & Forer, 1967; Lane & Treherne, 1970; Newcomb, 1969; Shay, 1972; Steer & Newcomb, 1969; Tamura, 1971). Their general appearance is clearly similar to other forms of tubules generally termed microtubules (Heath et al. 1970; Hepler & Fosket, 1971; Lane & Treherne, 1970; Ledbetter & Porter, 1963; Newcomb, 1969; O’Brien, 1967; Porter, 1966; Schnepf & Deichgräber, 1972; Tilney, 1971; Tucker, 1972; Yamada et al. 1971), but their difference in size necessitates further study to determine the class to which they belong.

The presence of microtubules in the Golgi apparatus is of particular interest since they possibly play a role in the functional processes of the Golgi apparatus. Microtubules would surely add an element of structural rigidity or anisotropy to the Golgi apparatus and could act to position dictyosomes within the cell or organize partially its internal structure. Microtubules might also serve to guide or to transport soluble precursors into or out of the Golgi apparatus in a manner similar to that in neurons and/or other cells (Behnke & Forer, 1967; Bikle, Tilney & Porter, 1966; Burton & Fernandez, 1973; Fernandez, Burton & Samson, 1971; Lane & Treherne, 1970; Ledbetter & Porter, 1963; Newcomb, 1969; Yamada et al. 1971) or to direct the movement of secretion vesicles from the Golgi apparatus.
REFERENCES


Golgi apparatus microtubules


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Figs. 1–4. Transverse sections through several dictyosomes showing the form and distribution of microtubules. The micrographs are oriented so that dictyosome maturation is depicted from top to bottom and arrows are included to mark tangentially sectioned microtubules. er, endoplasmic reticulum. Fig. 1, $\times 88,000$; Fig. 2, $\times 90,000$; Fig. 3, $\times 103,000$; Fig. 4, $\times 87,000$. 
Golgi apparatus microtubules
Figs. 5–7. Same as Figs. 1–4 except that microtubules are illustrated within cisternae and vacuoles (Figs. 5, 7) and the orientation of Fig. 6 is tangential to the average plane of the cisternae. Microtubules within the cisternae are much less prevalent than those in the intercisternal space. Arrows mark tangentially sectioned microtubules. Fig. 5, × 90,000; Fig. 6, × 72,000; Fig. 7, × 100,000.

Fig. 8. Microtubules, like those of the Golgi apparatus and of the contractile and accessory vacuoles, are often found within the endoplasmic reticulum, particularly in those segments of endoplasmic reticulum near the pellicle (arrows). × 100,000.
Golgi apparatus microtubules