Proc. Nat. Acad. Sci. USA
Vol. 69, No. 7, pp. 1669–1672, July 1972

Proventriculus of a Marine Annelid: Muscle Preparation with the Longest Recorded Sarcomere

(Z-band/paramyosin/electrophysiology/excitation–contraction and –relaxation coupling/syllid worm)

JOSÉ DEL CASTILLO*, MARGARET ANDERSON*, AND DAVID S. SMITH†

* Laboratory of Neurobiology, Medical Sciences Campus, University of Puerto Rico, San Juan, P.R. 00905; and
† Papanicolaou Cancer Research Institute and Department of Medicine, University of Miami, Miami, Florida 33136

Communicated by Daniel Mazia, April 17, 1972

ABSTRACT **Each muscle cell of the syllid (Annelida: Polychaeta) proventriculus, the region of the gut posterior to the pharynx, contains a single zigzagging Z band, flanked on each side by a sequence of I-A-H-A-I bands defined by thick (60–90 nm) and thin (5 nm) filaments. The thick filaments show a 14-nm periodicity similar to paramyosin. The muscle cell terminates at the level of the outer I bands. The muscle does not include a complete sarcomere in the strict sense, as defined by a pair of Z bands, but the equivalent H-H distance reaches about 40 μm. Electrophysiological evidence suggests that contraction and relaxation of the cell are, respectively, associated with depolarizing and hyperpolarizing potentials.**

The proventriculus of syllid worms is a tubular organ, composed primarily of myoepithelial cells, most of which are arranged radially between the lumen and the external surface. In the resting state the lumen of the proventriculus is collapsed, but upon contraction of the muscular wall it opens to allow water and food particles to be sucked in via the mouth. Relaxation occludes the lumen and expels the contents of the organ. Contraction and relaxation act in concert; peristaltic waves travel in a caudal direction, and valves at each end of the proventriculus ensure unidirectional flow. In its mechanical function, the syllid proventriculus resembles the nematode esophagus (1).

The special interest of this syllid preparation lies in the fine structure and organization of the myoepithelial cells, which were described by Haswell (2) as striated with a sarcomere repeat that, while not visible with the naked eye, "can be made out with a pocket lens magnifying four or five diameters." Haswell found that these muscle cells possess from one to several Z bands (on a species-specific basis), which define sarcomeres of up to 30 μm—10- to 15-times the length of sarcomeres of conventional vertebrate striated muscle. Haswell's observations were confirmed by Malaquin (3) and Okada (4), while Schmidt (5) described in *Syllis blomstrandi* isotropic and anisotropic bands—the I and A bands of modern terminology—that alternate radially in cross sections of the proventriculus wall.

A recent fine structural study of the proventriculus of *Syllis amica* (6) revealed arrays of thick and thin myofilaments, but striation was apparently absent; the author accordingly considered this tissue a smooth muscle in which the thick filaments resemble, in their large diameter and transverse periodicity, paramyosin filaments of molluscan adductor muscle. The present study of *Syllis spongiphila*, however, clearly reveals a striated muscle in which the conventional terminology for the bands, including the single Z band of each cell, is appropriate. A preliminary description of this finding has been published (7).

MATERIALS AND METHODS

Specimens of *Syllis spongiphila* were collected for this study in mangrove swamps of Northeast Puerto Rico and the Bay of San Juan, and were kept in the laboratory in shallow dishes of aerated sea water. For electron microscopic studies, the proventriculus was fixed in 2.5% glutaraldehyde–0.05 M cacodylate buffer (pH 7.4)–14% sucrose. After 4 hr of fixation, the material was placed for 1.5 hr in similarly buffered 1% OsO_4. After dehydration in an ethanol series, material was embedded in Araldite (CIBA, Duxford, England). Sections were cut with glass knives on an LKB Ultrotome III, stained with salts of lead and uranium, and examined in a Philips EM 200. For electrophysiological work, worms were pinned on a translucent Silicone rubber platform in a plastic chamber, and the body wall was cut open to expose the proventriculus. Natural sea water at 24° was used as the bathing solution in all experiments. Electrical recording was conducted with conventional KCl-filled glass microelectrodes inserted into cells from the external aspect of the organ.

RESULTS AND DISCUSSION

Fig. 1*a* represents a light micrograph of a transverse section of the proventriculus of *Syllis spongiphila* in which the radially arranged columnar muscle cells are cut longitudinally. A dark band concentric with the lumen is seen equidistant from the internal and external surfaces. This line represents the series of aligned Z bands of the adjacent cells. In this specimen, which is oriented on its side in the micrograph, the cells close to the dorsal and ventral surfaces of the proventriculus are about 50 μm in length, lengthening to over 100 μm in the lateral regions of the tube. A longitudinal section of a pair of cells from an intermediate position is illustrated in a survey electron micrograph in Fig. 1*b*. This field includes the inner and outer fiber insertions, respectively, on the border of the lumen and on the outer surface of the proventriculus. The zigzagging Z band is clearly defined, but the remaining sarcomere pattern is only faintly visible in this plane of section, and becomes interpretable only by the aid of transverse profiles of the fiber that reveal the myofilament arrangement. On each side of the Z band extends a single sequence of I-A-H-A-I bands, and the fiber inserts at the level of the outer I bands. A profile of an I band adjacent to the Z band is shown

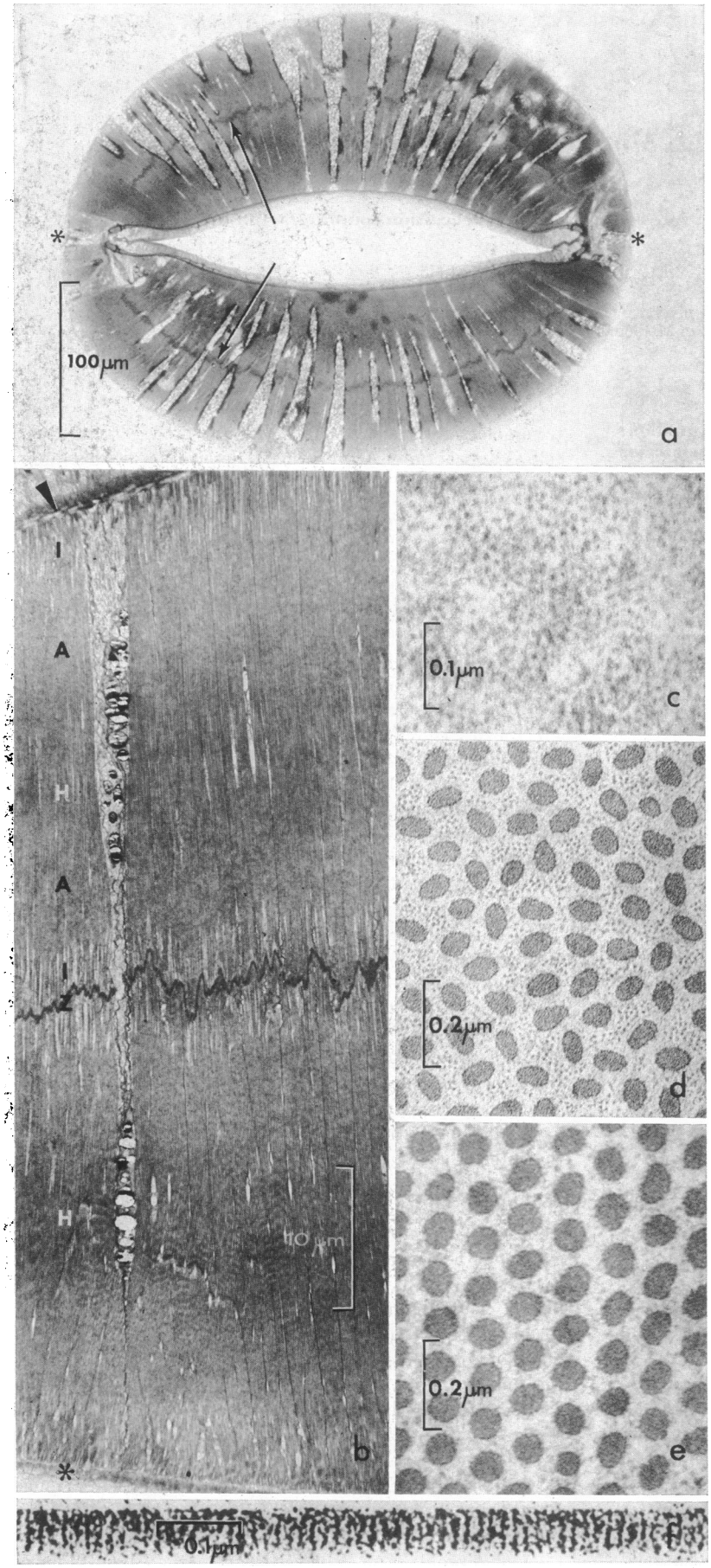

FIG. 1*a*. Transverse section of the proventriculus of *Syllis spongiphila*. Each of the radial myoepithelial cells possesses a single median Z-band (*arrows*). Regions of sarcoplasm containing the fiber nuclei and mineralized deposits alternate with the contractile cylinders. This muscle was fixed in partially contracted condition, and the lumen of the tube is seen. *Asterisks* indicate the dorsal–ventral axis of the proventriculus, as situated within the body cavity.

FIG. 1*b*. Survey electron micrograph of myoepithelial cells in the same plane of section as Fig. 1*a*. The inner (*arrow*) and outer (*) insertions of the fibers are seen, together with the median zigzagging Z band. The levels of I-, A-, and H-bands on either side of the single Z band are indicated.

FIGS. 1*c*, *d*, and *e*. Electron micrographs of transversely sectioned proventriculus muscle. In the I-band (1*c*), thin filaments alone are present; in the A-band (1*d*), thick and thin filaments are present in an interdigitating array, while the H-band (1*e*) is characterized by thick filament profiles.

FIG. 1*f*. A longitudinally sectioned thick filament showing an about 14-nm periodicity. The contrast has been enhanced *via* a Xerox copy of the original micrograph.

in Fig. 1*c*; this region contains only thin (5 nm) filaments, presumably of actin. The thick filaments are 60–90 nm in diameter, and circular in cross-section in the H zone (Fig. 1*e*); they become elliptical in cross-section and taper in the A bands where they are associated, as in other striated muscles, with thin filaments (Fig. 1*d*). The characteristics of the thin filament orbitals of the A region have not been established, but preliminary counts indicate a thin/thick ratio above 12:1. Paradoxically, the H zone, of lower electron density than the A band in conventional muscles, is here rendered more opaque than the A band by the greatly increased diameter of the thick filaments. The basic sarcomere repeat of striated muscle is the Z-to-Z distance: the muscle described above contains only a single Z band and thus does not include a complete sarcomere in the strict sense. However, the corresponding H-H distance in the proventriculus muscle is over 30 μm in Fig. 1*b*, and reaches about 40 μm in the lateral fibers in *Syllis spongiphila*.

An electron micrograph of a longitudinally sectioned thick filament is shown in Fig. 1*f*. In this material, the repeat period of the transverse banding is about 14 nm. This length corresponds closely with that found in paramyosin of molluscan adductor muscle (8, 9), but not with the periodicities of 77 or 115 Å reported in another species of *Syllis* (6).

These preliminary findings establish the presence of a striation pattern almost twenty times the length of the sarcomeres of vertebrate skeletal muscle, and twice that of the longest invertebrate sarcomere hitherto studied by electron microscopy (10).

Preliminary studies have been conducted on the electrophysiological mechanisms that control the contraction–relaxation cycle. The myoepithelial cells of the proventriculus have a resting potential of -49.5 ± 7.8 mV ($n = 83$). Spontaneous activity of various degrees occurred in most preparations. Commonly, small potentials were observed, up to 15 mV in amplitude, that were characterized by a fast initial phase and a slower decay from the peak. These spontaneous potentials, which resemble synaptic potentials, occurred as either hyperpolarizations or depolarizations. The small potential changes were not accompanied by noticeable movement of the proventriculus. Occasionally, large positive deflections of up to about 50 mV were recorded (Fig. 2*a*). Sometimes, large, complex, and relatively long-lasting spontaneous discharges, frequently associated with visible contractile activity of the proventriculus, took place. These bursts of activity appear to be the result of summation of many depolarizing and hyperpolarizing potentials. Fig. 2*b* and *c* illustrate two series of discharges associated with coordinated peristaltic waves. The activity is initiated by depolarizing potential changes that build up to a peak, which, as shown in Fig. 2*b*, can reach, and even overshoot, the zero reference level. The peak is followed by an abrupt drop to the resting level, and a hyperpolarizing phase that is made up of discrete negative-going potentials. Direct visual observations indicate that the depolarizing phase is associated with contraction of the myoepithelial cells and with opening of the lumen, while the hyperpolarizing phase is associated with muscle relaxation and occlusion of the lumen. The electrical characteristics of the spontaneous potential changes have not been elucidated. The depolarizing potentials resemble excitatory synaptic potentials; because they can build up to overshoot the baseline, there is the possibility that a regenerative response of the membrane may be involved.

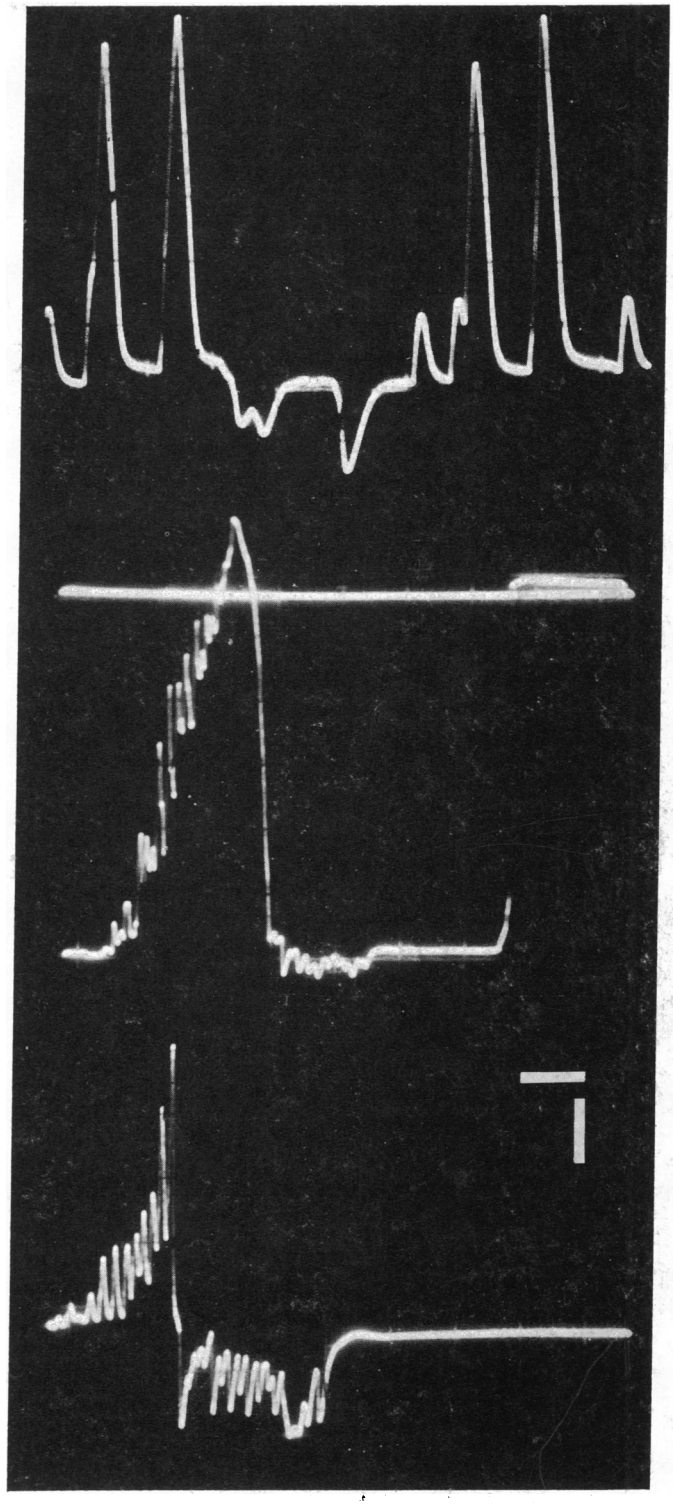

FIG. 2. Spontaneous electrical activity recorded from single myoepithelial cells of a syllid proventriculus. *Top*, simple depolarizing and hyperpolarizing potential changes. The rapidly rising depolarizations of large amplitude were only occasionally observed. *Middle* and *bottom*, complex potential changes associated with peristaltic waves of contractile activity. A depolarizing phase, made up of many positive-going potential changes, is followed by a hyperpolarizing phase, made up of negative-going potential changes. In the *middle*, the electrode was removed from the cell to indicate the zero reference level. Vertical calibration, 10 mV; horizontal calibrations 0.2 sec (*top*) or 1.0 sec.

In summary, this muscle offers a unique set of features of interest to the physiologist and to the morphologist. The syllid proventriculus appears to possess a relaxation mechanism that is associated with negative-going potential changes; hitherto only the pharynx of *Ascaris* has revealed such a mechanism (1). For the first time, a myofilament possessing a periodicity resembling that of paramyosin has been found in a striated muscle and, in addition, the unusual sarcomere length of the syllid myoepithelial cell offers a new level of resolution to experimental approaches to a single unit of the contractile system.

Supported (JC) by USPHS NIH Grants NS-07464 and K6N-14938 and (DSS) N.S.F. Grant GB-12117. We thank Mr. F. MacKenzie for collecting the specimens, Dr. E. Gijón for his help with some of the electrophysiological experiments, and Miss E. Lindgren for her help in preparing the micrographs. This is contribution no. 33 from the Laboratory of Neurobiology.

1. del Castillo, J. & Morales, T. (1967) *J. Gen. Physiol.* **50,** 603–629.
2. Haswell, W. A. (1890) *Quart. J. Microsc. Sci.* **30,** 31–50.
3. Malaquin, A. (1893) *Mem. Soc. Sci. Lille Ser. 4,* **18,** 1–477.
4. Okada, Y. K. (1928) *Quart. J. Microsc. Sci.* **72,** 219–245.
5. Schmidt, W. J. (1935) *Z. Zellforsch. Mikrosk. Anat.* **23,** 201–212.
6. Wissocq, J. C. (1970) *7th Int. Congress of Electron Microscopy,* Grenoble, 801.
7. Smith, D. S. & del Castillo, J. (1971) Abstr., Amer. Soc. Cell Biol., *J. Cell Biol.* 279.
8. Hanson, J. & Lowy, J. (1957) *Nature* **180,** 906.
9. Hanson, J. & Lowy, J. (1960) in *The Structure and Function of Muscle,* ed. Bourne, G. H. (Academic Press, New York & London), pp. 265–335.
10. Hoyle, G. (1970) *Sci. Amer.* **222,** 85–93.