Similar paracrystals of endoplasmic reticulum in the photoemitters and the photoreceptors of scale-worms

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Summary. Paracrystals of tubular endoplasmic reticulum are the characteristic of the luminous cells in the elytra of polynoïd worms, and they are the sources of luminescence as well as of fluorescence. Structurally similar paracrystals are now found to be the main constituent of the lens in the eyes of all the Aphroditidae.

Tubules of endoplasmic reticulum of uniform diameter (200-250 Å) and precisely disposed in a sinuous and repetitive mesh (figure 1) have been occasionally encountered in an extremely broad diversity of organisms. Our list of 52 references which is certainly not exhaustive, includes examples from plant cells[1-3], invertebrate[4-7], vertebrate[8] and even pathogenic instances[9,10]. The areas of paracrystalline endoplasmic reticulum (PER) are generally scarce, small (0.1-1.0 µm) and inconstant. Their function remains enigmatic. We describe here 2 instances in which PER is considerably more abundant and significant. Both occur in scale-worm (Annelida, Aphroditidae), a group in which several species, belonging to the subfamily Polynoïnae, are bioluminescent[11]. PER is the striking feature of the photogenic cells of the elytra[12] and, as shown below, its function in bioluminescence can be demonstrated. It is also found in unusually large aggregation in the eyes, where it constitutes the greater part of the lens.

The photogenic area of the elytrum forms a part of the single layer of epithelial cells on the lower face. Within each photogenic cell are found 20-30 sharply delimited areas of PER, which, since they can be proved to be the light-emitting entities, we have termed photosomes[18]. These are polyhedral, 3-10 µm across; tubules situated at the edges often run parallel to the plasma membrane, establishing with it a 'dyade' suggestive of electrotonic coup-

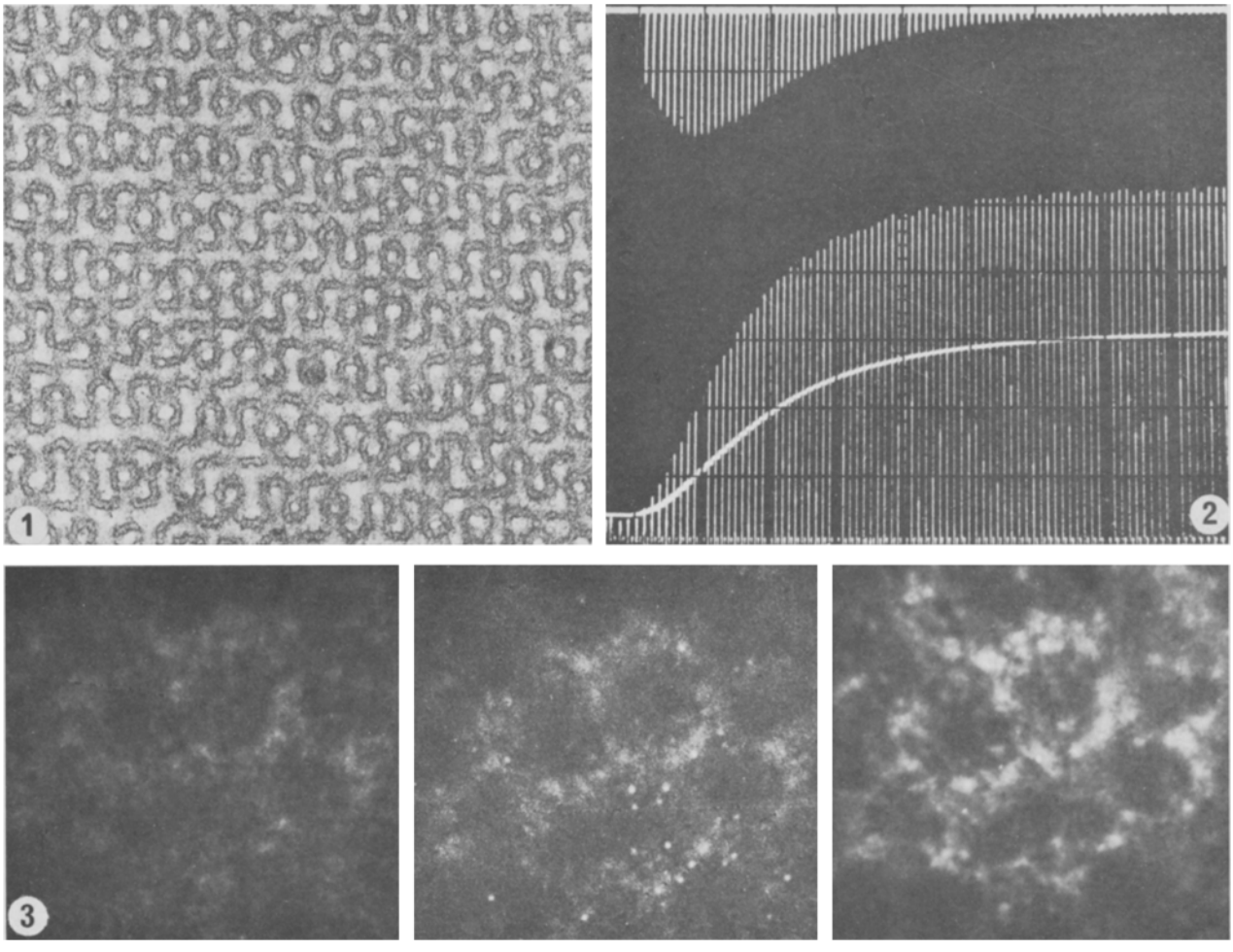

Fig. 1. Paracrystalline endoplasmic reticulum. Photosome of a luminous cell of *Acholoe astericola.* × 78,000. Fig. 2. An isolated elytrum of *Harmothoe lunata* is stimulated with electrical shocks (5 msec, 3 V) repeated every sec. Each shock (lower line, dots) elicits a luminous response (upper line, relative intensity directed downward). The continuous curve is the integration of the amount of luminescence emitted. The lower line shows successive measurements of fluorescence intensity taken between each flash. After adjustment to the same scale, curves of fluorescence and integration are superimposable. Fig. 3. Same field of the photogenic area, in a living elytrum of *Acholoe astericola,* photographed through microscope and image intensifier. Left: initial fluorescence. Middle: autophotography in luminescence. Right: consecutive fluorescence. Active photosomes form polygonal patterns which delimit the cells. Background noise appears in the middle picture as white dots, the exposure time being 4 sec while 10 msec are enough for fluorescence pictures. × 750.

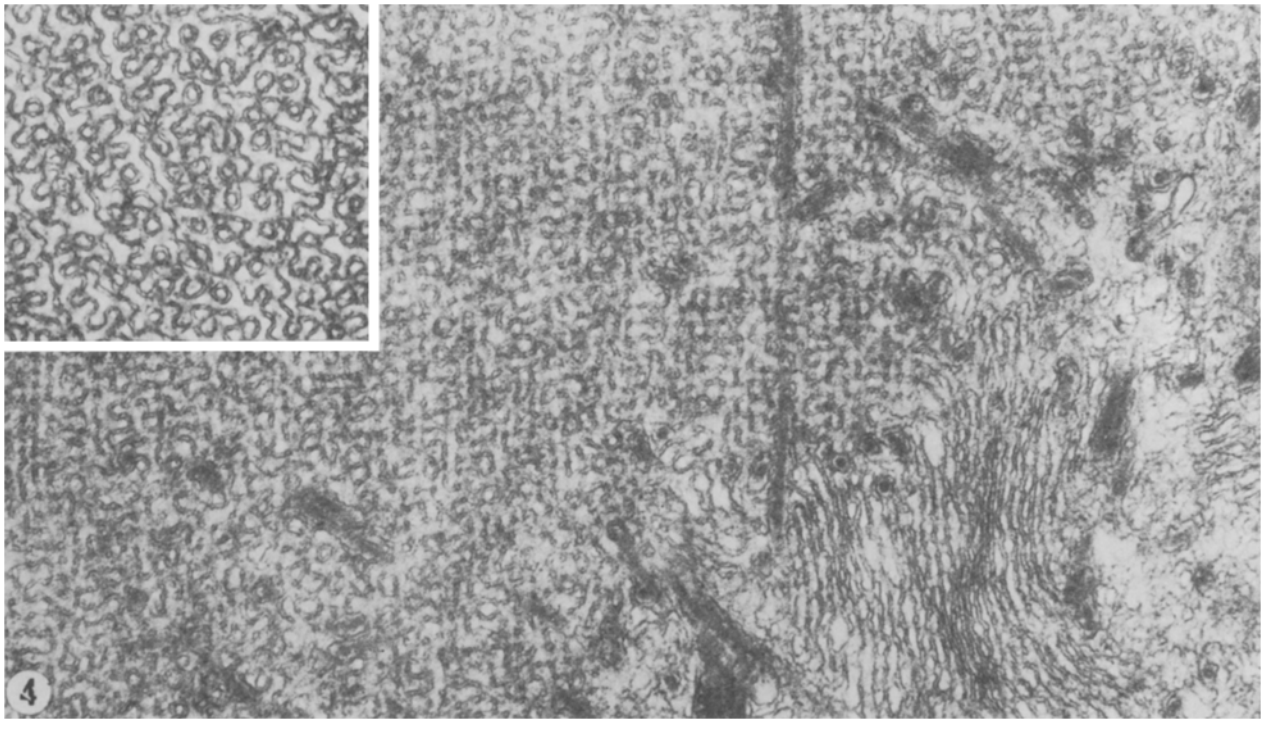

Fig. 4. PER in the lens of *Lagisca extenuata*. Although the plane of section differ from the one of figure 1, the fundamental architecture is the same. × 36,000. Insert × 40,000.

ling[13]. Photosomes are acidophilic, rich in glycoproteins and in cysteine, and devoid of nucleic acids[14]. In vivo, they usually exhibit a green fluorescence, due to a flavin, whose spectral maximum of 525 nm is identical with that for bioluminescence[15]. We observed photosomes in all the luminous species of polynoids so far studied: *Acholoe astericola*, *Harmothoe impar*, *H. longisetis*, *H. lunulata*, *Gattyana cirrosa*, *Lagisca extenuata*, and *Polynoe scolopendrina*.

In all these examples, bioluminescence can be evoked in isolated elytra by electrical stimulation; the basic response to a single shock is a flash. Repetitive stimulation at 0.5 or 1 Hz, stabilized just above threshold, elicits a corresponding series of unitary responses exhibiting typical and progressive variations. In particular, the intensities of the successive flashes first increase, then slowly decrease until the system is exhausted, the overall variation being exactly expressed by a difference of exponentials. Records of fluorescence taken between each flash indicate that fluorescence intensity, which remains stable at rest, is a function of the amount of luminescence previously emitted (figure 2).

Owing to their flatness and transparency, whole isolated elytra may be observed under a fluorescence microscope equipped with image intensification. Quiescent photosomes are individually and specifically revealed by their fluorescence, while observation during a flash identifies them as the sources of luminescence (figure 3). It can also be seen that the photosome population of each elytrum does not respond as a whole to the stimulus, the facilitation which occurs during the early stages of emission being instead due to a progressive recruitment of active units[17]. This coupling process, which explains the variability of the flashes, occurs centripetally within each photocyte, as well as centrifugally over the entire photogenic area[18].

In the eyes of the same annelids, the lens is primarily composed of PER ultrastructurally identical to that found in the photocytes. Singla[19] briefly mentioned this unusual architecture in the eye of *Arctonoe vittata*; we now extend this observation to all the Aphroditidae studied in this respect which include, in addition to the 7 luminous Polynoïds listed above, the nonluminous polynoids *Halosydna gelatinosa*, *Lepidonotus squamatus* and *Scalisetosus pellucidus*, the sigalionids *Sigalion mathildae* and *Sthenelais boa* and the hermionid *Hermione hystrix*. These animals usually have 4 eyes, covered by the first pair of elytra. The eyes are small, cupule-shaped and of typical rhabdomeric structure. The proximal layer is made up of somata of photoreceptive and pigment cells, while the central part of the eye, roughly spherical and about 50 µm in diameter, comprizes the lens. This body is in turn composed of numerous lens elements, each limited by a plasma membrane and linked by gap junctions. Each element, about 6–10 µm in size, represents the apical process of a pigment cell and is filled with membranous arrays. The principal and central part is paracrystalline while, at the periphery, the tubules of endoplasmic reticulum become disorganized or arranged in parallel saccules resembling myeloid bodies (figure 4). Among them are found long bundles of an opaque and filamentous substance, but no other organelle. The paracrystals of the lens are neither luminescent nor fluorescent.

Lenses are known to be very diverse in Annelids[20]. Apart those made by special cells[21,22], they usually derive from pigment cells, which either secrete a hyaline material[23] or develop slender interdigitated apical processes[24,25]. The PER-containing lenses undoubtedly constitute a new type, apparently characteristic of the whole family Aphroditidae.

Conclusion. The peculiar substructure of PER permits the development, within the cell, of a considerable membranous surface, as well as the segregation of 2 compartments. In the case of photosomes, it is clear that this membrane is excitable, the response being a flash and that the compartments hold the reactive substances. The functional significance of PER in the lens is far more difficult to understand. Electroretinograms, recently recorded by A. Morille[26] on several polynoïds, show two types of responses, one being

probably related to the lens. But the hypothesis, following which the paracrystals of the eyes could be attuned to some parameter of the bioluminescent emission would not explain the existence of a similar lens in nonluminous species. Nevertheless, it remains puzzling to observe, in the same animals, an unusual organelle exceptionally developed in the 2 organs which have to do with light.

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Protein digestion in the sea gooseberry *Pleurobranchia bachei* A. Agassiz (Ctenophora: Tentaculata)[1,2]

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Summary. Peaks of proteolytic activity of pharyngeal juice occur at pH 5.75 and pH 7.5. The proteases responsible for digestion include a tryptic alkaline protease and a thiol-activated acid protease which is probably cathepsin B. Levels of proteolytic activity parallel those of other carnivorous invertebrates which feed on zooplankton.

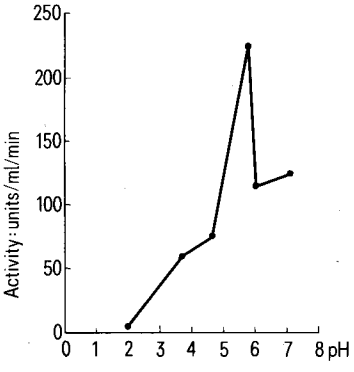
Pleurobrachia bachei is a small, ovoid, comb jelly which preys on a wide range of marine zooplanktors by means of bizarre sticky papillae borne on its tentacles called colloblasts or 'lasso cells'. Food-ladened tentacles are typically thrust into the comb jelly's mouth where pharyngeal juices elicit prey release followed by rapid digestion.

There is little information available on protein digestion in the Ctenophora[4], and nothing is known about the proteases involved. In this report, we have examined proteolytic action on the pharyngeal juice of *P. bachei* and obtained a preliminary characterization of the enzymes involved. Our studies in protein digestion have necessarily emphasized acid proteases. This is due to a) the very limited quantity of digestive juice available to us for analysis and b) its acid range pH (about pH 5.3).

Several hundred *P. bachei* were obtained by plankton net at depths of 1-20 m from Indian Arm, Vancouver, British Columbia. Pharyngeal juices were carefully extracted by inserting a 3-mm length of 0.38 mm inner diameter by 1.09 mm outside diameter Intramedic polyethylene tubing into the comb jelly's foregut and applying vacuum using a µl syringe. Typical yields of digestive juice per individual were 2-5 µl. Samples were pooled and immediately quenched in liquid nitrogen followed by freezer storage at −30 °C.

Proteolytic activity of 2 0.3-ml lots of pharyngeal juice was estimated[5] employing serum albumin as a substrate. Digests were run at 37 °C. Freed amino acids were determined at 280 nm in microcuvettes in a Hitachi-Perkin-Elmer spectrophotometer[6]. A pH profile was obtained over a range of pH 2-8, using universal phosphate buffer[7]. The following activators and inhibitors were applied to the extracts to obtain a partial characterization of the proteases involved: 0.008 M cysteine (cathepsin B and C activator); 0.001 M iodoacetamide (cathepsin B inhibitor); 1% trypsin egg-white inhibitor; 0.23% L-1-tosylamide-2-phenyl-ethylchloromethyl ketone (TPCK: chymotrypsin inhibitor); 0.23% N-a-p-tosyl-L-lysine chloromethyl ketone (TLCK: trypsin inhibitor).

The pH profile of the 1st sample (figure) shows a prominent peak between pH 5 and 6: 225 units at pH 5.75 and the indication of a 2nd peak above pH 7: 125 units per ml at pH 7.1. The 2nd sample which was tested showed greater activity: 420 units/ml at pH 5.6 and 416 units/ml at pH 7.5. TPCK chymotrypsin inhibitor had no inhibitory effect at pH 7.5. TLCK trypsin inhibitor caused a 70% reduction in activity at pH 7.5. Trypsin egg-white inhibitor caused a 98% reduction in activity at pH 7.5. Cathepsin B and C activator cysteine caused a 90% increase in activity at pH 5.6. Iodoacetamide caused a 60% reduction in activity at pH 5.6. These results indicate the presence of a tryptic protease and a thiol-activated acid protease which appears to have cathepsin B properties. Chymotrypsin, the enzyme which has been found in Cnidaria[8] does not appear to be present on the basis of the effect of chymotrypsin inhibitor. In-



Effect of pH on proteolytic activity of *Pleurobrachia* pharyngeal juice.