TUNICATA AND CEPHALOCHORDATA

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Keywords: Protochordata, Tunicata, Ascidiacea, Thaliacea, Larvacea, Cephalochordata, ascidians, sea-squirts, pyrosomes, salps, doliolids, appendicularians, amphioxus, tunic, endostyle, coloniality, filter-feeding

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Summary

Tunicata and Cephalochordata, collectively named Protochordata, are filter-feeding marine animals that, as invertebrate members of the phylum Chordata, share with vertebrates the basic chordate characteristics.

Tunicata are found at all latitudes and can assume a planktonic or benthic lifestyle. Coloniality is widespread and a larval stage followed by metamorphosis is the rule in the class Ascidiacea. Blooms of pelagic tunicates are quite common in warm seasons: their ecological relevance relies on the key role played by these animals in the alimentary chain of open seas.

Cephalochordata are small, fish-like animals living in sandy bottoms, of interest for

evolutionary studies.

1. Introduction to Protochordata: General Characteristics

Chordata represents the major phylum among Deuterostomes. Members of the phylum share bilateral symmetry and at least four anatomical characteristics: (i) the presence of a notochord in the form of an incompressible elastic dorsal rod, which prevents shortening of the body when longitudinal muscles contract; (ii) a hollow dorsal nerve cord, somewhat modified at the front end; (ii) a ciliated pharynx provided with gill slits; (iv) a muscular tail (post-anal part of the body).

The phylum Chordata comprises about 45 000 species distributed in three subphyla: Tunicata (Urochordata), Cephalochordata, and Vertebrata. In adult vertebrates, the notochord is either surrounded or (partially or totally) substituted by a vertebral axis made of repeating cartilaginous or osseous pieces called vertebrae.

Invertebrate Chordata represent about 3% of the total chordate species and are collectively named Protochordata. Unlike vertebrates, which are widely distributed on land, freshwater, and seawaters, and are mainly active predators or grazers, Protochordates are filter-feeding marine animals, most of them characterized by a sedentary lifestyle.

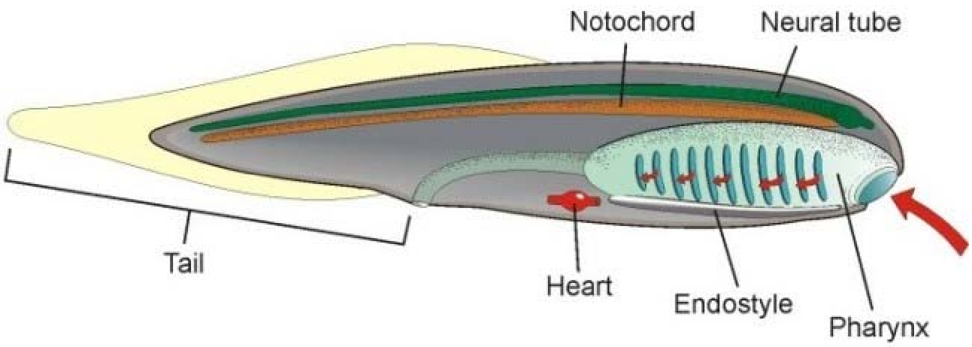

Figure 1. General characteristics of a primitive chordate. Water enters the pharynx through the mouth (large arrow) and exits through the pharyngeal slits (small arrows)

2. Tunicata

Tunicates or Urochordates ($o\upsilon\rho\alpha$ = tail; $\chi o\rho\delta\eta$ = rope, cord) are marine, filter-feeding animals, with solitary or colonial forms, benthic and pelagic. They usually present a muscular tail provided with a notochord and a hollow dorsal nerve tube only in larval stages. The pharynx is well developed in the adult and generally occupies most of the body volume. Tunicates owe their name to the test or tunic—the peculiar covering in which the adult body is embedded. They traditionally include three classes: Ascidiacea, Thaliacea, and Larvacea.

2.1. Ascidiacea

Ascidians ($\alpha\sigma\kappa\iota\delta\iota o\nu$ = small bag) or sea-squirts are sessile, marine invertebrates diffuse throughout the world, mainly in shallow tropical and temperate waters. About 3000 species have been reported so far. Both solitary and colonial (compound) species of ascidians may be found. Individuals or zooids range from 0.5 mm to 20 cm in length, those of colonial species being much smaller than solitary ones. In colonial ascidians, zooids share a common tunic and are frequently interconnected by a common circulation. In this case, zooids can be either partially isolated but connected by stolons (deriving from the body wall and containing connective tissue and, sometimes, blood vessels) or partially or entirely embedded in the common tunic.

2.1.1. Tunic

Figure 2. The solitary ascidian, Ciona intestinalis, a common organism in the marine substrate. The oral and cloacal siphons are marked by large and small arrows, respectively (courtesy of Dr. R. Brunetti).

The ascidian body is covered with a tunic, acting as a sort of flexible exoskeleton. The adult tunic consists of an amorphous matrix, rich in water and salts, and a fibrous component, which often contains cells, mainly composed of tunicin, a cellulose-like polysaccharide, and proteins crosslinking tunicin fibers. The tunic, the consistency of which varies from a soft sheet to a leather-like covering, is produced by the external epithelium (epidermis) of the body wall (mantle), and is frequently supplied with blood vessels and cells, probably deriving from the circulation, some of which are presumed to be involved in tunic synthesis. The tunic anchors the animal to the substrate, offers protection and support, and is frequently encrusted with foreign materials such as sand, algae, and other ascidians. It may also play an excretory role as, in some cases, it accumulates nephrocytes. In some species, it contains calcareous secretions (spicules) of various shapes. Many species contain symbiotic algae within the tunic. *Prochloron* is noteworthy as it is a prokaryotic symbiont of some colonial ascidians and contains photosynthetic pigments usually found in green algae. Epibionts are easily found in the ascidian tunic of both solitary and colonial species in the form of protists, algae, sponges, bryozoans, cnidarians, mollusks, annelids, and crustaceans.

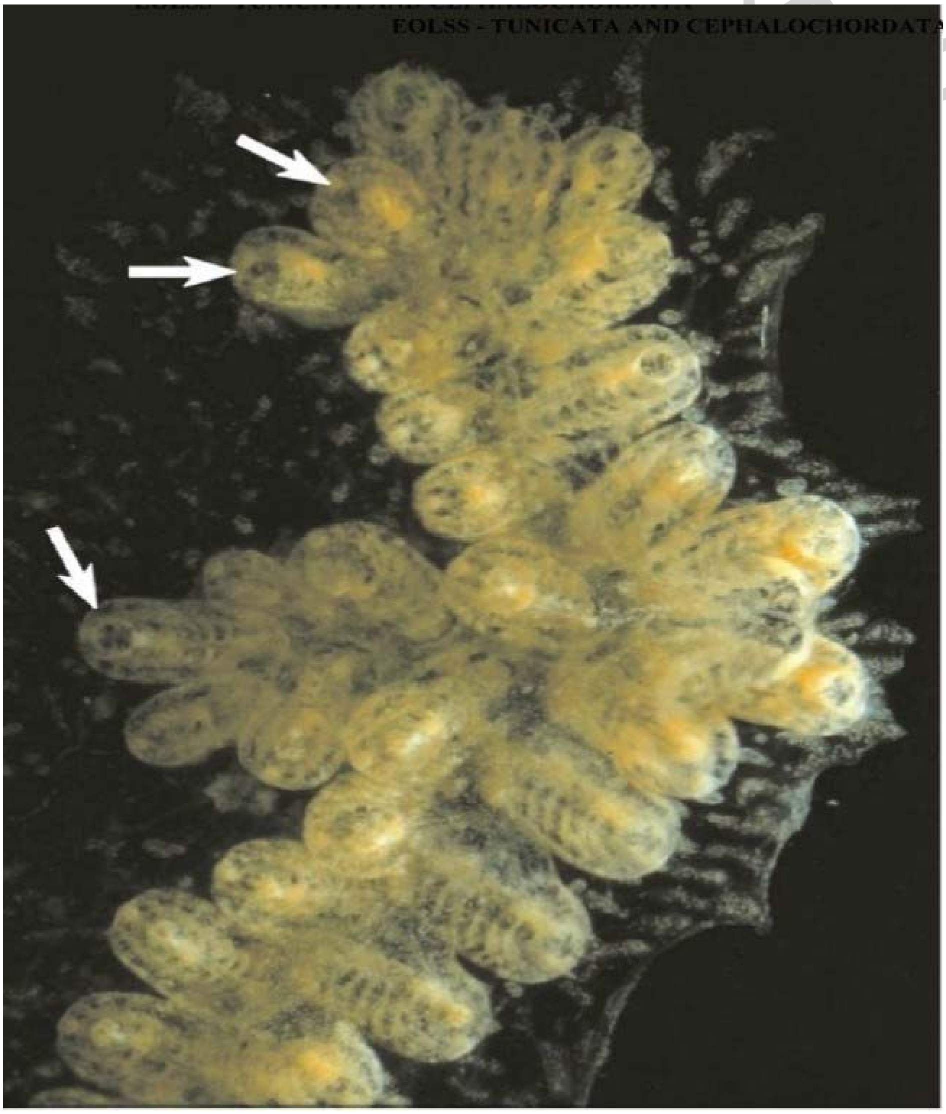

Figure 3. The compound ascidian, Botrylloides leachi. All the zooids are produced through asexual reproduction. They are embedded in a thin common tunic and filter seawater entering through oral siphons (arrows).

2.1.2. Morphology and Physiology

The hollow, sac-like adult body opens to the outside through two siphons. A terminal oral or inhalant siphon allows water to enter the anterior part of the digestive tract, the pharynx, or the branchial basket, which is greatly enlarged and perforated by numerous gill slits or stigmata. Each branchial fissure is bordered by rings of cilia that beat synchronously and, forcing water from the pharynx lumen to the peribranchial (atrial) cavity, create the incoming water flow. The atrium or peribranchial cavity is defined by the body wall and opens to the exterior through the dorsal atrial (or exhalant or cloacal) siphon. Expelled water is enriched in digestive wastes and, eventually, gametes. The pharynx is widely surrounded by the atrium, being attached to the body wall below the ring of oral tentacles, which prevent large objects from entering the branchial basket, along the midventral line and on its external surface through vascularized trabeculae. Midventrally, on the bottom of the basket, is the endostyle, a ciliated glandular groove that produces a mucous net and probably also digestive enzymes. Opposite the endostyle, along the pharynx dorsal line, a ciliated ridge, called the dorsal lamina, is found. The endostyle is homologous to vertebrate thyroid and its cells can fix iodine. Iodoproteins are components of the mucous net, which is continuously secreted by the endostyle, and slides on both sides of the pharyngeal wall toward the dorsal lamina. Particulate material entering the branchial basket is caught by the sticky endostylar mucous net and carried to the dorsal lamina, where the net is rolled into a mucous strand which is then conveyed to the esophagus. The transport of the mucus is driven by cilia distributed along the internal wall of the branchial basket, often located on protruding papillae or bars. Periodic squirting, that is sudden muscular contractions of the body with vigorous ejection of water through the siphons, helps to clean the stigmata of debris and/or mucus and to completely renew pharyngeal water contents.

The post-pharyngeal digestive tract is U-shaped, and consists of esophagus, stomach, and intestine, the esophagus forming the proximal descending arm, and the intestine the distal ascending arm. The stomach, between esophagus and intestine, is a sac-like enlargement of the digestive tube, with a smooth or folded wall. A network of vesicles and tubules forms the pyloric gland that surrounds the posterior part of the gut. Tubules converge into small ducts that join, in turn, to form a common duct through which secretions are released into the stomach, close to the intestine. Like vertebrate liver, the pyloric gland can accumulate glycogen; however, the characteristics and role of these secretory products in digestion are still uncertain.

Ascidians have a well-developed, open (haemocoelic) vascular system. Blood circulates in mesenchymatic sinuses and lacunae, in the mantle and pharyngeal walls, and inside vessels, defined by epidermis extensions, in the tunic. In some species, contractile blind swellings (ampullae) mark the end of tunic vessels.

The heart is a double-walled tube, with an outer pericardium and an inner myocardium, deriving from invagination of the outer wall. The two sheets define a narrow pericardial cavity, considered the only remnant of the ascidian coelomic cavity. The heartbeat occurs in the form of contractile waves from one end of the tube to the other. Excitation centers exist at each end of the myocardium, and heartbeat reversal usually occurs every two to three minutes.

Ascidian blood consists of a colorless plasma, isotonic with seawater, and a wide variety of blood cells (haemocytes) that can be grouped into at least four categories, such as undifferentiated cells (haemoblasts and lymphocyte-like cells), phagocytes (hyaline amoebocytes and uni- or multivacuolar macrophage-like cells), cytotoxic vacuolated cells (granular amoebocytes and morula cells), and vacuolated storage cells (pigment cells and nephrocytes). Haemocytes take part in immunosurveillance, histocompatibility, tunic synthesis, excretion, and budding.

Blood plays a minor role in respiratory exchanges and contains no respiratory pigments. Since most of the tissues are in close contact with water, most exchanges occur by simple diffusion. In any case, the pharynx may be a good candidate for the main respiratory organ.
Ascidians lack true excretory organs. Being aquatic animals, they release their nitrogen wastes into outflowing water in the form of ammonia, by diffusion through the pharyngeal wall. However, uric acid and urates also are produced, and these compounds are usually stored, as crystals, inside the vacuoles of nephrocytes and released upon the death of the organism. Renal sacs, in the form of vesicles protruding from the body wall, occur in some species: they contain uric acid precipitates, symbiotic bacteria and fungi, and are filled with a liquid isotonic with seawater. The renal sacs are not comparable to vertebrate kidney, and their actual function remains unclear.

Pigment cells contain pigments of various natures (caronenoids, flavins, melanins) within their vacuoles. They and the nephrocytes may accumulate in specific areas of the body (e.g., tunic, branchial sac, parts of the mantle near siphons) thus giving zooids their typical pigmentation. Extreme variability in color may be observed in some colonial ascidians within the same species. In *Botryllus schlosseri*, the inheritance of pigments is genetically controlled. Symbiotic algae and spicules also may contribute to pigmentation patterns.

The nervous system of the adult body consists of a cerebral ganglion located in the connective tissue between the two siphons and surrounded by a connective fibrous sheath. Neural cell bodies are arranged in several layers in the outer cortex, around a fibrous medulla. Nerves arise from the two ends of the ganglion and spread through the mantle to siphons, mantle musculature, and digestive system. In some species, ablated ganglia can be regenerated completely in a few weeks.

A neural gland, the function of which remains obscure, is always closely associated with the neural ganglion, located ventrally, laterally, or dorsally to the ganglion itself. Like the ganglion, it originates from the embryonic neural tube but lacks nerves, and its lumen, through a ciliated duct and a ciliated funnel, opens into the pharyngeal cavity at the base of the oral siphon. Cilia in the duct create an incoming water flow from the pharynx to the gland lumen. A dorsal strand connects the neural gland with the gonads: it is a narrow tube emerging from the lumen of the neural gland, associated with a dorsal strand plexus of visceral neurons. Various roles have been attributed to the neural gland, especially neurosecretory and endocrine. Based on its embryological origin, neurogenic activity, and relationship with the cerebral ganglion, the neural gland may be homologous with the neurohypophysis in vertebrates.

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Bibliography

Bone Q., ed. (1998). *The Biology of Pelagic Tunicates*. Oxford: Oxford University Press. [A comprehensive review of the anatomy and biology of Thaliacea and Larvacea.]

Burighel P. and Cloney R. (1997). Urochordata: Ascidiacea. *Microscopic Anatomy of Invertebrates: Hemichordata, Chaetognatha, and the invertebrate Chordates*, Vol. 15 (ed. F.W. Harrison and E.E. Ruppert), 221–347. New York: Wiley-Liss. [A comprehensive review of the microscopic anatomy of Ascidiacea.]

Kott P. (1985). The australian Ascidiacea part 1, Phlebobranchia and Stolidobranchia. *Memoirs of the Queensland Museum*, **23**, 1–440.

Kott P. (1990). The australian Ascidiacea part 2, Aplousobranchia (1). *Memoirs of the Queensland Museum* **29**, 1–266.

Kott P. (1992). The australian Ascidiacea part 3, Aplousobranchia (2). *Memoirs of the Queensland Museum*, **32**, 375–620. [Three comprehensive guides to the anatomy, ecology, and taxonomy of Australian ascidians.]

Monniot C., Monniot F. and Laboute P. (1991). *Coral Reef Ascidians of New Caledonia.* Paris: Éditions de l'ORSTOM. [A guide to the diversity, ecology, and taxonomy of tropical ascidians, enriched with beautiful photographs.]

Ruppert E.E. (1997). Cephalochordata (Acrania). *Microscopic anatomy of Invertebrates: Hemichordata, Chaetognatha, and the invertebrate Chordates*, Vol. 15 (ed. F.W. Harrison and E. E. Ruppert, 349–504. New York: Wiley-Liss. [A comprehensive review of the microscopic anatomy of Cephalochordata.]

Biographical Sketches

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