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bHLH proneural genes as cell fate determinants of entero-endocrine cells, an evolutionarily conserved lineage sharing a common root with sensory neurons

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ABSTRACT

Entero-endocrine cells involved in the regulation of digestive function form a large and diverse cell population within the intestinal epithelium of all animals. Together with absorptive enterocytes and secretory gland cells, entero-endocrine cells are generated by the embryonic endoderm and, in the mature animal, from a pool of endoderm derived, self-renewing stem cells. Entero-endocrine cells share many structural/functional and developmental properties with sensory neurons, which hints at the possibility of an ancient evolutionary relationship between these two cell types. We will survey in this article recent findings that emphasize the similarities between entero-endocrine cells and sensory neurons in vertebrates and insects, for which a substantial volume of data pertaining to the entero-endocrine system has been compiled. We will then report new findings that shed light on the specification and morphogenesis of entero-endocrine cells in *Drosophila*. In this system, presumptive intestinal stem cells (pISCs), generated during early metamorphosis, undergo several rounds of mitosis that produce the endocrine cells and stem cells (ISCs) with which the fly is born. Clonal analysis demonstrated that individual pISCs can give rise to endocrine cells expressing different types of peptides. Immature endocrine cells start out as unpolarized cells located basally of the gut epithelium; they each extend an apical process into the epithelium which establishes a junctional complex and apical membrane specializations contacting the lumen of the gut. Finally, we show that the *Drosophila* homolog of *ngn3*, a bHLH gene that defines the entero-endocrine lineage in mammals, is expressed and required for the differentiation of this cell type in the fly gut.

1. Introduction

1.1. Sensory neurons and entero-endocrine cells: an evolutionary perspective

Sensory neurons and endocrine cells lining the intestinal epithelium share many functional, structural and developmental properties. Sensory neurons, in particular gustatory neurons, and entero-endocrine cells express the same or related types of receptors on their apical membrane (Rozengurt and Sternini, 2007; Mace et al., 2015; Gribble and Reimann, 2016); upon stimulation, transmitters are released basally which, in case of gustatory neurons, synaptically depolarize the target neurons, or, in entero-endocrine cells, reach their target tissues via the blood stream or local diffusion. The structural and functional similarity is reflected in highly conserved molecular pathways controlling the development of neurons and entero-endocrine cells, and may hint at a deep evolutionary relationship between the two cell systems at the base of the metazoa. Basal metazoa lacking a nervous system or muscular system, including porifera and placozoa, possess cells with the characteristics of endocrine or exocrine gland cells. For example, in placozoa, gland cells with secretory granules, expressing proteins that form part of the synaptic compexes in higher animals (synapsin, syntaxin, SNAP-24), as well as neuropeptides (FMRFamide), are integrated in the ciliated epithelium that forms the body wall (Smith et al., 2014; Fig. 1A, B). Structurally similar secretory cells were described in larvae of the sponge *Amphimedon*, where they mount a Ca-response to environmental stimuli that is involved in larval settling and metamorphosis (Nakanishi et al., 2015).

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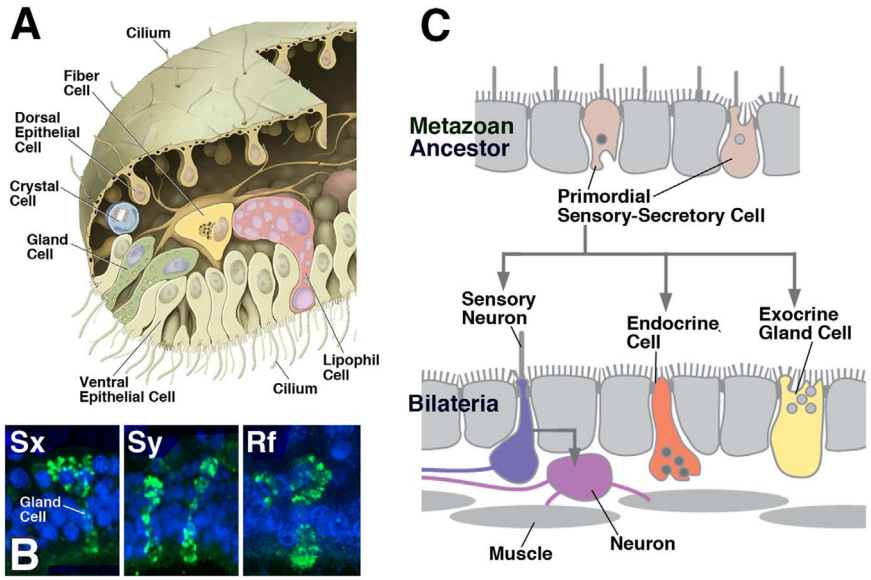

Fig. 1. (A) Cutaway diagram of placozoan, showing ciliated epithelial cells, gland cells and other cell types (from Smith et al. (2014), with permission). (B) Immunohistochemical detection of synaptic proteins [syntaxin (sx); synapsin (sy)] and peptide FMRFamid (Rf) in placozoan gland cells (from Smith et al. (2014), with permission). (C) Schematic depicting hypothetical evolutionary origin of bilaterian neurons and enterocendocrine. The shared characteristics between these cell types can be interpreted as "deep homologies", inherited from a hypothetical type of sensory-secretory cell already present in basal metazoan.

It is conceivable that such specialized epithelial cells, which have acquired sensory, neurosecretory and effector properties, could constitute the "protoneurons" that were present already in basal metazoans, and that gave rise to both neurons and endocrine cells in the bilaterian animals.

As schematically depicted in Fig. 1C, both outer (ectodermally derived) and inner (endodermally derived) epithelia of bilaterians are accompanied by a layer of muscle cells and neurons. In the intestine, neurons form an enteric plexus (called stomatogastric or pharyngeal nervous system in many invertebrate taxa); secretory gland cells and entero-endocrine cells are integrated in the intestinal epithelium. Endocrine cells, enteric neurons, and groups of neurons in the brain express a large number of phylogenetically related transmitters/hormones, called "brain-gut peptides", in addition to being interconnected by nerve fibers. Taken together, these elements form a complex integrated system, the "brain-gut axis", that controls feeding behavior and digestive functions, including motility of the gut, secretion of enzymes, and resorption and excretion of nutrients, ions and water (for recent review, see Latorre et al. (2016), Gribble and Reimann (2016)).

In the following, we will survey recent research data pertaining to the structure, function and development of the entero-endocrine system. Outside several vertebrate models, a good amount of knowledge has accumulated in various arthropods. The similarities that exist in enteroendocrine cells and sensory neurons in regard to their structure, expression of peptides/signaling pathways, and, in particular, the developmental mechanisms by which they are specified, are compatible with the idea of a shared origin of these cell types.

1.2. Comparison of the structure and function of the entero-endocrine system in insects and vertebrates

Entero-endocrine cells are specialized, endodermally derived cells with a cell body located basally, and a neck that reaches towards the luminal surface of the epithelium (Fig. 2A3). According to recent serial electron microscopy studies (Bohórquez et al., 2014, 2015), entero-endocrine cells project elongated basal processes, so called neuropods, that interact with glial cells and neurons of the enteric plexus (Fig. 2B). Neurons of the enteric plexus originate from the neural crest and migrate towards the gut; in a similar fashion, enteric (stomatogastric) neurons of insects are generated in the foregut ectoderm and undergo migration to reach their destination in the intestinal wall (Fig. 2A1).

Endocrine cells possess two regulated pathways of secretion which are structurally defined by large dense core vesicles (LDCV) and synaptic-like microvesicles (SLMV; Rindi et al., 2004). Dense core vesicles have an electron-dense interior and measure 80–100 nm; they are regularly associated with the storage and release of neuropeptides. Microvesicles resemble the small synaptic vesicles (20–40 nm) releasing classical transmitters of neurons, such as acetyl choline, at the synaptic cleft. In enteroendocrine cells, both types of vesicles are targeted to the basal cell membrane, and released into the interstitial space surrounding enteric neurons/glia and capillaries, or, in case of insects, the open hemolymph space.

The cellular mechanisms controlling stimulus reception, vesicle trafficking and docking, as well as the released peptides themselves, are very similar in entero-endocrine cells and sensory neurons. Typical neuronal markers like N-CAM, synaptophysin, or vesicular monoamine transporter, are also found in entero-endocrine cells, where they perform the same or similar functions. Thus, the docking of vesicles, as well as the transport and re-uptake of transmitters utilize conserved molecular pathways. Importantly, also the gustatory G-protein-coupled receptors expressed in sensory neurons of the mouth cavity and tongue. Numerous members of the T1R family (sensing of sugars and L-amino acids) and T2R (bitter tastes) are expressed in the intestine, and could be assigned to numerous types of entero-endocrine cells (Rozengurt and Sternini, 2007). Similarly, signal transducers, including Gα-gustducin and transducing 2, were also found to be expressed in these cells. Stimulation of G-protein-coupled receptors results in an increase in intracellular calcium, and functional studies confirmed that tastants applied to entero-endocrine-derived cell lines caused a Ca-spike, and led to a release of peptides, including cholecystokinin (CKK; from STC-1 cells stimulated with bitter tastants) and glucagon-like peptide (GLP-1; from GLUTag cells stimulated by sweet tastants) (Wu et al., 2002; Jang et al., 2007). "Visceral behavioral" studies, using flat sheet preparations of live rodent colon, confirmed the direct role of entero-endocrine gustation in controlling gut motility (Kendig et al., 2014). Monosodium glutamate (MSG), a stimulant of the T1R1/T1R3 umami receptor, when applied to the colon preparations, elicited a peristaltic reflex, which was abolished in preparations from mice that were mutant for T1R1. Interestingly, aside from enteroendocrine cells, another specialized type of vertebrate intestinal cell called "tuft cell"