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Minireview

Conserved genetic pathways controlling the development of the diffuse endocrine system in vertebrates and *Drosophila*

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ABSTRACT

The midgut epithelium is formed by absorptive enterocytes, secretory cells and endocrine cells. Each of these lineages is derived from the pluripotent progenitors that constitute the embryonic endoderm; the mature midgut retains pools of self-renewing stem cells that continue to produce all lineages. Recent findings in vertebrates and *Drosophila* shed light on the genetic mechanism that specifies the fate of the different lineages. A pivotal role is played by the Notch signaling pathway that, in a manner that appears to be very similar to the way in which Notch signaling selects neural progenitors within the neurectoderm, distinguishes the fate of secretory/endocrine cells and enterocytes. Proneural genes encoding bHLH transcription factors are expressed and required in prospective endocrine cells; activation of the Notch pathways restricts the number of these cells and promotes enterocyte development. In this review we compare the development of the intestinal endocrine cells in vertebrates and insects and summarize recent findings dealing with genetic pathways controlling this cell type.

1. The diffuse endocrine system (DES): a brief overview

Digestive function, including motility of the gut, secretion of enzymes, resorption of nutrients, ions and water, is regulated by two systems, the autonomic nervous system, and the endocrine system. In vertebrates, the latter is formed by specialized endocrine glands, in particular the pancreas, as well as scattered endocrine cells integrated in the intestinal wall. These cells, which outnumber all other endocrine organs by a wide margin, form the diffuse endocrine system (DES). Within the DES, at least 14 different cell types have been identified which produce many different peptide hormones with a specific regional distribution (for review, see Rehfeld, 1998; Montuenga et al., 2003; Rindi et al., 2004). For example, secretin, produced in the duodenum, was one of the first hormones discovered and characterized around the turn of the 20th century (reviewed in Modlin et al., 2006); released by gastric acid, secretin stimulates secretion of bicarbonate-rich pancreatic juice. Other well characterized DES hormones are gastrin (produced in the stomach) and cholecystokinin (CKK; produced in the small intestine). Release of gastrin is triggered by protein rich food and in turn increases acid secretion from parietal cells; likewise, CKK, triggered by fats and proteins, stimulates the secretion of pancreatic enzymes and gall bladder contraction.

Enteroendocrine cells are elongated, epithelial cells with a cell body located basally, and a neck that reaches the luminal surface of the epithelium (“open endocrine cells”; Fig. 1A and B). In other cases, the apical contact to the lumen is lost (“closed endocrine cells”). Both types of endocrine cells are characterized by two regulated pathways of secretion which are morphologically defined by large dense-core vesicles (LDCV) and synaptic-like microvesicles (SLMV; Rindi et al., 2004). Vesicles are targeted to the basal cell membrane and the hormones are released into the interstitial space or into capillaries. With regard to the cellular mechanisms controlling vesicle trafficking and docking, as well as the hormones themselves, enteroendocrine cells share many characteristics with neurons, a theme that will reoccur when looking at development (see below). For example, typical neuronal markers like N-CAM, synaptophysin, or vesicular monoamine transporter, are also found in enteroendocrine cells.

Open enteroendocrine cells exist in all animals, from cnidarians to vertebrates. The DES of insects has been studied in considerable detail, and its complexity, in terms of number of different hormones produced and the control of hormone release, is comparable to that of vertebrates (for review, see Zitnan et al., 1993; Veenstra et al., 2008; Veenstra, 2009; Winther and Nässel, 2001). As in vertebrates, the peptide hormones found in insect enteroendocrine cells also occur as neurotransmitters in neurons of the central nervous system and stomatogastric nervous system (comparable to the vertebrate autonomic nervous system) and are therefore frequently referred to as “brain-gut peptides” (Fujita et al., 1981). For example, the peptides of the tachykinin family are found both in DES cells of the midgut (Fig. 1C–E), as well as in neurons. Local tachykinin release from neurons has spatially restricted effects on

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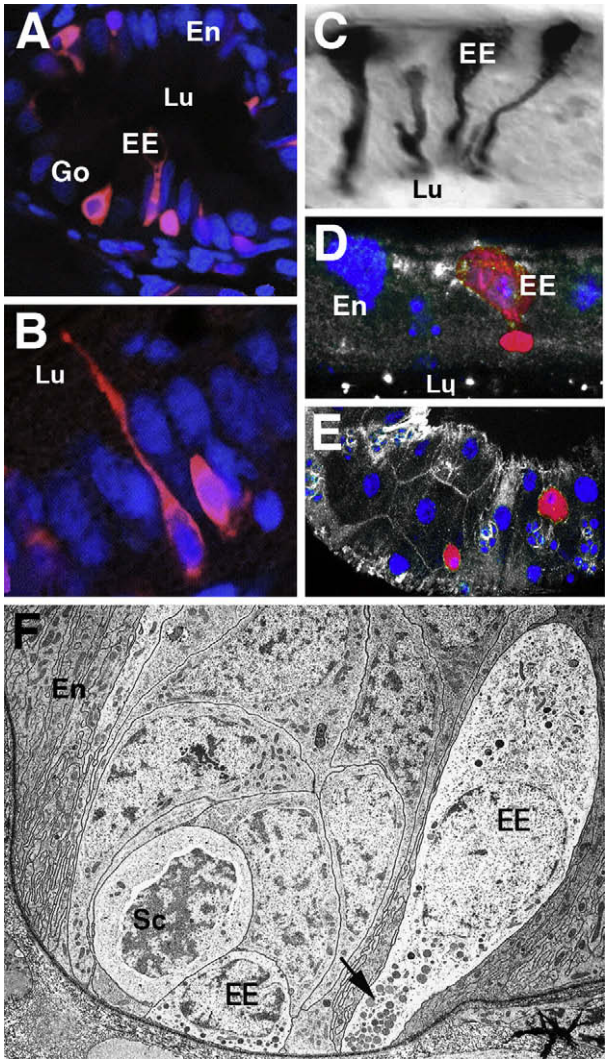

Fig. 1. Enteroendocrine cells in the vertebrate and insect intestine. (A and B) Five day zebrafish posterior intestine (from Crosnier et al., 2005). Enteroendocrine cells (EE) are labeled by monoclonal antibody 2F11 (red; nuclei of all cells labeled by TOPRO-3 in blue) exhibit an elongated, neuron-like shape, with a basal cell body and a slender apical process integrated into the enterocyte layer (En) and contacting the gut lumen (Lu). Exocrine goblet cells (Go) are also labeled. (C) Endocrine cells in locust midgut, labeled by antibody against locust tachkinin-related peptide (from Winther and Nässel, 2001). Note characteristic shape and position of cells, resembling vertebrate enteroendocrine cells. (D) Cross section of *Drosophila* adult midgut epithelium. Enteroendocrine cell labeled by anti-tachykinin antibody (red). Cell nuclei labeled with Sytox (blue). As in locusts, endocrine cell body is located basally and possesses a club-shaped apical protrusion. (E) Tangential section of *Drosophila* adult midgut epithelium, showing scattered distribution of tachykinin-positive endocrine cells (red). (F) Electron micrograph of basal portion of midgut epithelium, showing enteroendocrine cells (EE) in close spatial association with proliferating stem cell "nests" (Sc; from Lehane, 1998). Note dense-core vesicles near basal membrane of endocrine cells (arrow). (For interpretation of color mentioned in this figure the reader is referred to the web version of the article.)

muscle contractility; systemic release into the hemolymph (insects have an open circulatory system, with a blood-like hemolymph filling the body cavity) acts on many effector organs, including the excretory Malpighian tubules, the heart, and the somatic musculature (Winther and Nässel, 2001). The storage and systemic release of peptide hormones involves dense-core vesicles located at the basal membrane of the cell (Fig. 1F). Peptides of the FMRFamide, myosupressin and leucomyosuppressin family act on the visceral musculature (inhibition of midgut muscle tone; Lange and Orchard, 1998) and secretory cells of the midgut (stimulation of digestive enzyme release; Fusé et al., 1999). Many other brain-gut peptides have been identified (for review see Veenstra et al., 1995, 2008; Veenstra, 2009); as for vertebrate DES-derived hormones, the parameters of release and physiology of most of these peptides have not yet been elucidated.

2. Development of enteroendocrine cells in vertebrates

It was known for a long time that the autonomic neurons populating the intestinal wall and ganglia associated with it arise in the neural crest and migrate to their final destination during embryogenesis (Anderson et al., 2006). Several decades ago, the hypothesis was put forward that enteroendocrine cells, given their strong similarities with neurons, were also derived from migrating cell populations originating in the neural crest (reviewed in Modlin et al., 2006). Subsequent investigations (Pictet et al., 1976) showed convincingly that that is not the case, and that, instead, enteroendocrine cells segregate from within the same endodermal primordium that gives rise to the enterocytes of the gut epithelium. More recently, using appropriate markers it was possible to study how the different cell lineages (endocrine cells, exocrine secretory cells, enterocytes) relate to each other, and what molecular mechanisms control their fate.

At an early stage of development, the endoderm forms an epithelial tube in which all cells are mitotically active (Henning et al., 1994; Crosnier et al., 2005; Fig. 2A, B and E). As the gut tube increases in surface area, the epithelium is gradually folded into the villi and crypts that are characteristic of the gut. At that point, proliferation becomes restricted to the crypts, and cells in the villi undergo differentiation (Fig. 2C and F). Eventually, proliferation settles into its adult pattern, in which a small number of slowly dividing stem cells populate the crypts; next to the crypts, at the base of the villi, cells that were produced by the stem cells enter a phase of fast proliferation ("transient amplifying progenitors"); moving further apically, towards the tip of the villi, cells become postmitotic and differentiate (reviewed in Hauck et al., 2005; Fig. 2D, G and H). At the villus tip is a region where old and/or damaged cells, including enterocytes and endocrine cells, are sequestered and undergo apoptosis (Potten and Allen, 1977). In this way, there is a constant streaming of cells from the crypts where they are born upward into the villi where they differentiate and eventually die.

The first enteroendocrine cells first appear at an early stage of development before the gut epithelium has formed villi and crypts. Expressing markers for endocrine fate (Math-1; Ngn-3; for review, see Lee and Kaestner, 2004; Schonhoff et al., 2004a) these cells seem to be the first ones to become postmitotic; surrounding enterocyte progenitors continue to divide. Also at later stages, when the characteristic spatio-temporal pattern of proliferation has been set up (stem cells in crypts, transient amplifying progenitors at crypt-villus boundary), enteroendocrine cells are continuously produced. As in the embryo, cells committed to the endocrine fate often withdraw from the mitotic cycle earlier than presumptive enterocytes (Bjerknes and Cheng, 2006; Fig. 2I). Differentiating endocrine cells migrate apically into the villi, although their speed seems to be slower than that of enterocytes.

Much attention has been given to the question how enterocytes, endocrine cells and other secretory cell types are related. The most direct approach to address this question is to generate labeled clones. To this end, markers are activated in individual proliferating progenitor (or stem) cells. These markers are then inherited by all of the progeny of the labeled cell, thereby showing what different cell types are derived from the one individual progenitor. The analysis performed by Bjerknes and Cheng (2006) yielded