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Review article

Enteric nervous system development in avian and zebrafish models

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ABSTRACT

Our current understanding of the developmental biology of the enteric nervous system (ENS) and the genesis of ENS diseases is founded almost entirely on studies using model systems. Although genetic studies in the mouse have been at the forefront of this field over the last 20 years or so, historically it was the easy accessibility of the chick embryo for experimental manipulations that allowed the first descriptions of the neural crest origins of the ENS in the 1950s. More recently, studies in the chick and other non-mammalian model systems, notably zebrafish, have continued to advance our understanding of the basic biology of ENS development, with each animal model providing unique experimental advantages. Here we review the basic biology of ENS development in chick and zebrafish, highlighting conserved and unique features, and emphasising novel contributions to our general understanding of ENS development due to technical or biological features.

1. Introduction

It is clear from the scope of presentations at the 4th International Symposium on "Development of the Enteric Nervous System: Cells, Signal, Genes and Therapy", held in Rotterdam, The Netherlands (19–22 April, 2015) that active research using model systems is fuelled in equal measure by a fascination with the basic biology of the enteric nervous system (ENS) and the drive to model and unravel the genetic basis of ENS disease states.

The use of model systems to study ENS development is long-standing. For more than 20 years, studies in mouse have been critical to understand genetic control of ENS development and to model ENS diseases. However, these studies were preceded by work using other model systems, notably the chick. For example, study of avian systems initially described the neural crest origins of the ENS, and provided a framework for understanding phenotypes arising in newly generated mouse mutants. More recently, studies in these and other non-mammalian model systems, such as zebrafish, are being used to model ENS development and ENS diseases (Fig. 1). Technical innovations have meant that there has been an ever-increasing capacity to perform genetic analysis with these alternative systems, making them increasingly used and increasingly important.

In this review we will describe key features of ENS development in chick and zebrafish, and will highlight important similarities and differences between these systems and compare to mammalian systems. Novel contributions to our general understanding of ENS development made by studies in these model systems, especially when due to unique biological traits or technical capacities, will be emphasized. The unique experimental tools available in these different model organisms will be highlighted. Finally, we will consider the future scope for use of model systems, to more fully understand ENS biology and ENS disease states.

2. The chicken embryo as a model for ENS development

The avian embryo, and in particular the chicken embryo, has a 2000 year history in the study of animal development (Stern, 2004). The sustained use of this animal model can be attributed to a number of advantageous features including ready availability of fertilized chicken eggs, low cost and maintenance of eggs, rapid embryonic development, and easy access to the embryo within the egg for observations and experimental manipulations. Further, the chick embryo is a valid model to inform on human development since early embryonic chick and human morphology and development are very similar, and the chicken and human genomes have significant homology of approximately 60% (International Chicken Genome Sequencing, 2004). With these features in mind

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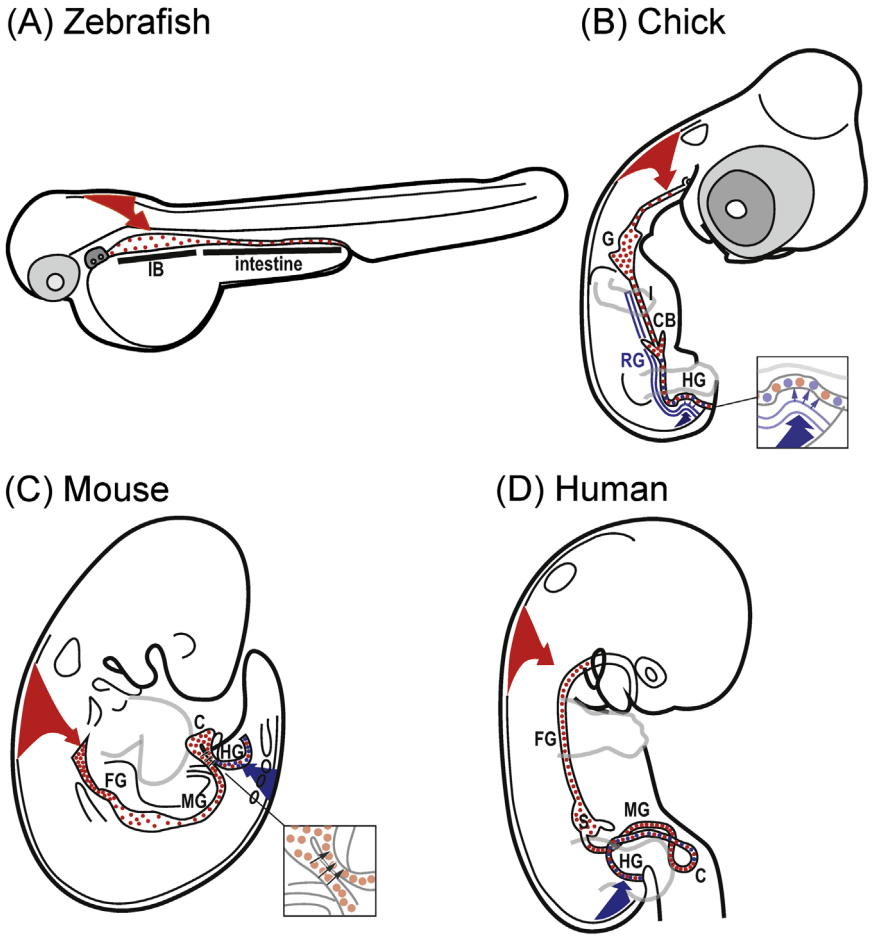

Fig. 1. Embryonic origins of the ENS in diverse vertebrate models. (A) The ENS of zebrafish derives from vagal NCC (red arrow) that enter the rostral gut tube at approximately 36 hours post-fertilization (hpf). Cells migrate caudally and progressively colonize the intestinal bulb (IB) (analogous to the stomach of mouse and human) and intestine. The gut is fully colonized by these vagal neural crest-derived ENS progenitors (red dots) by 66 hpf. As yet there is no evidence for any sacral contribution to the ENS in zebrafish. (B) In the chick, the ENS is formed primarily from vagal NCC at the level of somite 1–7 (red arrow) that enter the foregut (FG) at approximately embryonic day (E) 3–3.5 and migrate caudally to progressively colonize the gizzard (G) (mechanical stomach), intestine (I), cecal buds (CB) and hindgut, a process that is complete by E7.5 (red dots). Sacral NCC, arising caudal to somite 28 (blue arrow), also contribute to the ENS, first forming the extramural nerve of Remak (RG) (blue), and then migrating into the hindgut (inset, blue arrows) to colonize primarily the distal hindgut (blue dots). (C) The mouse ENS is formed principally from vagal NCC from the level of somite 1–7 (red arrow), which enter the foregut at approximately E9, and migrate caudally to colonize the foregut (FG), midgut (MG), cecum (C), and hindgut (HG) (red dots). In addition to rostrocaudal migration, trans-mesenteric migration of vagal NCC from the midgut to the hindgut also occurs (inset, arrows). Colonization of the length of the gut is complete by E14. An additional ENS contribution arises from NCC that migrate from the sacral region (caudal to somite 25) (blue arrow). These cells initially form pelvic ganglia adjacent to the hindgut, then migrate into the gut and primarily occupy the hindgut and caudal midgut (blue dots). (D) In the human, the ENS derives from vagal NCC (red arrow) that enter the foregut (FG) at 4 weeks of gestation and migrate along the gut to fully colonize the foregut, stomach (S), midgut (MG), cecum (C), and hindgut (HG) by week 7 (red dots). It is inferred, from mouse data, that sacral NCC also contribute to the hindgut ENS (blue hatched arrow), however no experimental evidence is yet available to confirm this. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

it is not surprising that many fundamental aspects of developmental biology such as neural crest migration and fate, limb patterning, neural tube patterning, somite segmentation, and left–right asymmetry have been elucidated using the chicken embryo (see Stern (2004)).

2.1. Early development and organization of the chick ENS

Like other vertebrates, the chicken gastrointestinal (GI) tract develops from a uniform tube-like structure where reciprocal epithelial-mesenchymal interactions pattern this organ into regions with specific morphologies and functions. Sonic hedgehog signals originating from the epithelium induce region-restricted expression of genes, such as homeotic genes (*Hox*, *Nkx*) and *Bmp4* in the mesenchyme, which in turn signals back to the epithelium to control patterning and differentiation along the anteroposterior (AP) axis (reviewed in Roberts et al. (1998)). Although grossly similar to the mammalian digestive system, the chicken GI tract has a number of key anatomical differences that are partly due to the fact that birds do not have teeth for the breakdown of food by chewing, but instead have mechanical breakdown within the digestive system. Thus the GI tract of the chicken comprises the esophagus, crop (temporary storage pouch), proventriculus (glandular stomach), gizzard (mechanical stomach), small intestine, ceca (paired blind pouches), and large intestine (colon). Another key difference is that avians have a cloaca, an orifice that serves as the single opening for the digestive, reproductive, and urinary tracts.

The chick embryo has been used for numerous pioneering studies on ENS development (Kuntz, 1910; Le Douarin and Teillet, 1973; Yntema and Hammond, 1954). Although Yntema and Hammond first identified a vagal neural crest origin for the ENS, Le Douarin and Teillet mapped the precise location of ENS precursors within the neural crest by using the now classical quail-chick grafting technique to selectively label regions of the neural axis. These authors demonstrated that the majority of the ENS precursors along the entire GI tract originate from neural crest adjacent to somites 1–7 (Le Douarin and Teillet, 1973). This and other