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The *Drosophila* blood-brain barrier as interface between neurons and hemolymph

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ABSTRACT

The blood–brain barrier is an evolutionary ancient structure that provides direct support and protection of the nervous system. In all systems, it establishes a tight diffusion barrier that hinders uncontrolled paracellular diffusion into the nervous system. In invertebrates, the blood–brain barrier separates the nervous system from the hemolymph. Thus, the barrier-forming cells need to actively import ions and nutrients into the nervous system. In addition, metabolic or environmental signals from the external world have to be transmitted across the barrier into the nervous system. The first blood–brain barrier that formed during evolution was most likely based on glial cells. Invertebrates as well as primitive vertebrates still have a purely glial-based blood–brain barrier. Here we review the development and function of the barrier forming glial cells at the example of *Drosophila*.

1. Introduction

In the past, research on the *Drosophila* model has disclosed many fundamentally important developmental processes. Not only the molecular and genetic code underlying axis determination is similar in flies and mammals but also paths to normal eye or heart development, metabolic control and finally disease progression obey evolutionary conserved mechanisms (Gonzalez, 2013; Nüsslein-Volhard and Wieschaus, 1980; Padmanabha and Baker, 2014; Qian and Bodmer, 2012; Rajan and Perrimon, 2013).

In addition, work on *Drosophila* and other invertebrate models has provided an enormous advance in our understanding of neuroscience. For example, the molecular strategies organisms use to define neuronal cell types appear evolutionary conserved. In all species analyzed so far, proneural genes promote neural development and all proneural genes known encode related bHLH transcription factors (Bertrand et al., 2002). Even the specification of distinct neuronal cell fates appears to require the activity of conserved transcription factors (Thor and Thomas, 2002). We are not surprised by the fact that all organisms rely on the very same set of main neurotransmitters. Likewise, synaptic function and the mechanisms underlying neuronal conductance are organized in very similar ways across the animal kingdom. Given the many conserved processes operating during neural development, it appears likely that more specific functional elements such as the blood–brain barrier are evolutionary conserved as well. Thus, the study of the *Drosophila* blood–brain barrier may have more general implications.

How and when did the blood–brain barrier appear during evolution of the metazoa? Obviously, neurons had to be formed first. In the simplest multicellular organisms with an epithelial organization, such as sponge- or cnidaria-like animals, special cells that were able to sense the environment emerged (Fig. 1). These cells were likely those that evolved into the first sensory neurons (Bullock and Horridge, 1965; Hartline, 2011). As more of these sensory neurons specialized, they gained a requirement of a direct support as well as protection by other cells. This is for example seen in photoreceptor neurons which always form together with supporting pigment cells and molecular data indicate that this pairwise appearance of sensory and support cell has emerged only once during evolution (Arendt, 2003; Gehring and Ikeo, 1999; Nilsson, 2004). Neuronal support cells then may have evolved into the glial lineage. However, although neuronal assistance may have evolved as the first glial cell task — a second, equally important glial function must have developed concomitantly. Since nurturing and protection by isolation are intimately coupled, glial cells formed an increasingly tight barrier allowing establishment of a constant ion milieu in the brain. Thus, we propose that early on glial cells were separated into the supportive glial cells within the CNS and an outer glial cell layer forming the barrier to the remaining body (Fig. 1).

Again, first signs of such supporting cells can be found in Cnidaria (Holtmann and Thurm, 2001a,b) although true glial cells cannot be seen in these animals (Hartline, 2011). Glial cells, however, are present in simple Acoela which originate before the split of protostomia and deuterostomia (Bailly et al., 2014; Bery et al., 2010; Hartline, 2011). Even in simple nematodes such as *Caenorhabditis elegans*, whose nervous system consists of only 302 neurons, glial cells forming a sheath

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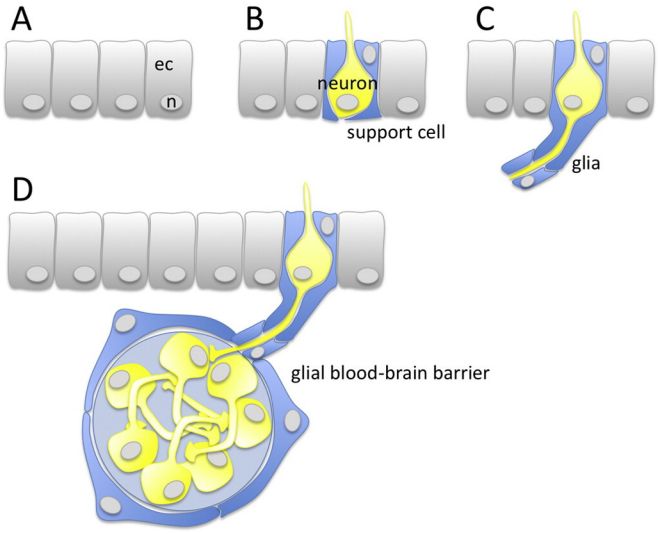

Fig. 1. Evolution of the blood–brain barrier. (A) Epithelia establish the outer surface of the animal. ec: epithelial cell, n: nucleus. (B) Within epithelia, sensory neurons developed that are accompanied by support cells. (C) Epithelial support cells evolved into glial cells. (D) Some neurons were transferred into the interior of the animal, where they were covered by a glial blood–brain barrier.

around the cephalic sensory neurons extend processes around the nerve ring that may serve as simple blood–brain barrier like structure (Oikonomou and Shaham, 2011; Stout et al., 2014).

A glial barrier around the nervous system is found throughout protostomia as well as in primitive deuterostomia. However, the blood–brain barrier had to change dramatically as vascularization developed. While the invertebrate nervous system floats in the hemolymph and thus just needs a tight barrier sheath, vertebrates developed a closed circulatory system. This implied that in the vascularized brain, all capillaries had to be "tight" in order to guarantee the insulation of the different compartments (body versus brain). Interestingly, in primitive vertebrates, such as in elasmobranch fish (sharks, skates, and rays), but also in some bony fish (sturgeon), the blood–brain barrier is still established by glial cells. These perivascular astrocytes form highly interdigitating lamellae without forming any tight junctions (Bundgaard and Abbott, 2008; Fig. 2). The barrier appears to be established by increasing the diffusion path between the perivascular glia. In mammals, the blood–brain barrier is formed by endothelial cells which – in the brain – are induced to form tight junctions by pericytes (Armulik et al., 2010; Daneman et al., 2010; Fig. 2). Astrocytes develop after the blood–brain barrier is established but their end-feet cover the entire endothelial/pericyte surface, where they are able to modulate blood–brain barrier properties (Abbott et al., 2006; Janzer and Raff, 1987; Mathiisen et al., 2010). In insects such as *Drosophila*, the blood–brain barrier is established by the perineurial and the subperineurial glial cells (Carlson et al., 2000; Stork et al., 2008; Figs. 2, 3). The subperineurial glial cells form so-called septate junctions which prevent paracellular diffusion just as the tight junctions in the mammalian endothelial blood–brain barrier. Interestingly, homologous proteins such as Claudin 5 are involved in the tightness of the barrier in flies as well as in mammals (Nitta, 2003; Stork et al., 2008) again pointing towards the evolutionary conservation of this structure.

In all animals, the blood–brain barrier separates the microenvironment around neurons and their processes from the remaining body fluids. This interface is also called neuro-vascular unit to highlight the intricate interaction of the two systems. The establishment of a barrier and the concomitant separation of brain and body compartments had immediate advantages for neuronal functionality, but also some disadvantages. On the one hand, the ion concentration in the brain can be kept at constant levels, which obviously helps to establish the sophisticated neuronal cross-talk that relies on minute changes in membrane potential. On the other hand, the separation of the nervous system from the remaining body called for the development of highly efficient and selective transport systems for metabolites and periphery derived signals. Thus, during evolution the formation of barrier functions coincides with the establishment of specific transport mechanisms. Based on evolutionary arguments, it can be anticipated that many of the relevant transport and relay mechanisms operating in the mammalian nervous system are already in place in the invertebrate blood–brain barrier. Here, we review the current knowledge on the invertebrate "neuro-vascular unit" which is an interface that not only controls brain homeostasis but also actively directs development and function of the nervous system.

2. The blood–brain barrier and the development of the *Drosophila* nervous system

In holometabolus insects, such as *Drosophila*, neurogenesis occurs in two phases. During embryogenesis the larval nervous system is generated by special stem cells called neuroblasts (Hartenstein and Wodarz, 2013). Their formation occurs in so-called proneural clusters specified by the balanced expression of proneural and neurogenic genes. To date, all embryonic neuroblast lineages have been identified and, from single cell tracing experiments, the trajectories of many of the neurons are known (Bossing et al., 1996; Jenett et al., 2012; Landgraf et al., 1997; Li et al., 2014; Rickert et al., 2011; Schmid et al., 1999; Schmidt et al., 1997; Urbach and Technau, 2003). Due to the advances in automated image analysis and 3D reconstruction programs, the establishment of a complete interaction map of the larval brain by serial electron microscopic analysis is in reach (Cardona et al., 2010; Sprecher et al., 2011).

About 10% of all neural cells in *Drosophila* are glial cells. The glial cells or subsets of them can be labeled using many different molecular markers (Halter et al., 1995; Klämbt and Goodman, 1991; Stork et al., 2008, 2012; Xiong and Montell, 1995). The developmental origins of

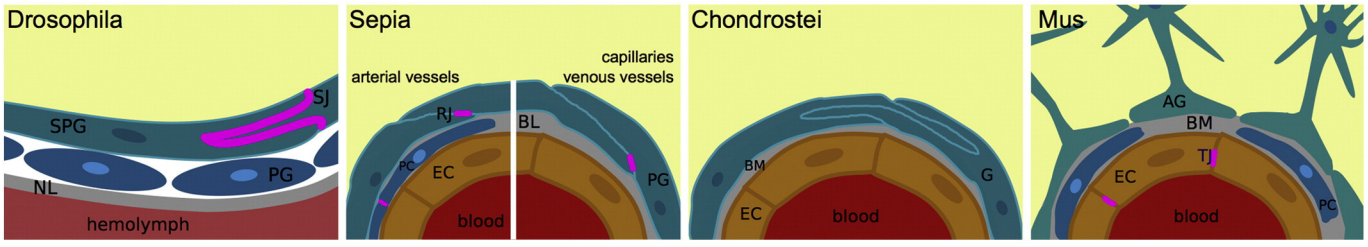

Fig. 2. The blood–brain barrier in invertebrates and vertebrates. Schematic view of the blood–brain barrier in *Drosophila*, sepia, sturgeon and the mouse. In *Drosophila*, a glial blood–brain barrier is found. It is built by two glial cell layers, the perineurial glia (PG) and the subperineurial glia (SPG), which form septate junctions (SJ) to prevent paracellular diffusion (NL: neural lamella). In sepia, restricting junctions (RJ) are formed between perivascular glial cells (PG) in capillaries and venous vessels and between pericytes (PC) in arterial vessels to prevent paracellular diffusion (EC: endothelial cells, BM: basal membrane). In contrast, in the sturgeon no occluding junctions are formed, but the overlap of the glial cells (G) abutting the endothelial cells (EC) is strongly increased to elongate the diffusion path and thereby prevent uncontrolled diffusion (BM: basal membrane). In the mouse, capillary-forming endothelial cells (EC) establish tight junctions (TJ) to seal the blood–brain barrier (BM: basal membrane, PC: Pericyte, AG: astrocytic glia).

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