Development of the Electric Organ in Embryos and Larvae

Introduction

Knifefish species endemic to Central and South America represent a highly useful model organism for the developmental study of electric organs. This biological adaptation allows the fish to generate weakly electric fields for electrolocation and electrocommunication within their immediate freshwater surroundings. By doing so, the fish sense their environment and connect with potential mates and competitors in deep water, low light conditions. The electric organs derive from one of two cell types during embryonic development. In this article, Alshami and colleagues were able to characterize the development of the electric organ in the Gymnotiform bluntnose knifefish, *Brachyhypopomus gauderio*. To do so, they skillfully employed an RMC Boeckeler PowerTome XL (PT XL) ultramicrotome (Fig. 2) to cut ultrathin sections of fixed specimens for electron and light microscopy analyses. This approach allowed the researchers to successfully determine where and when precursor cells originate, where and when they translocate, and the cell-type into which they develop.

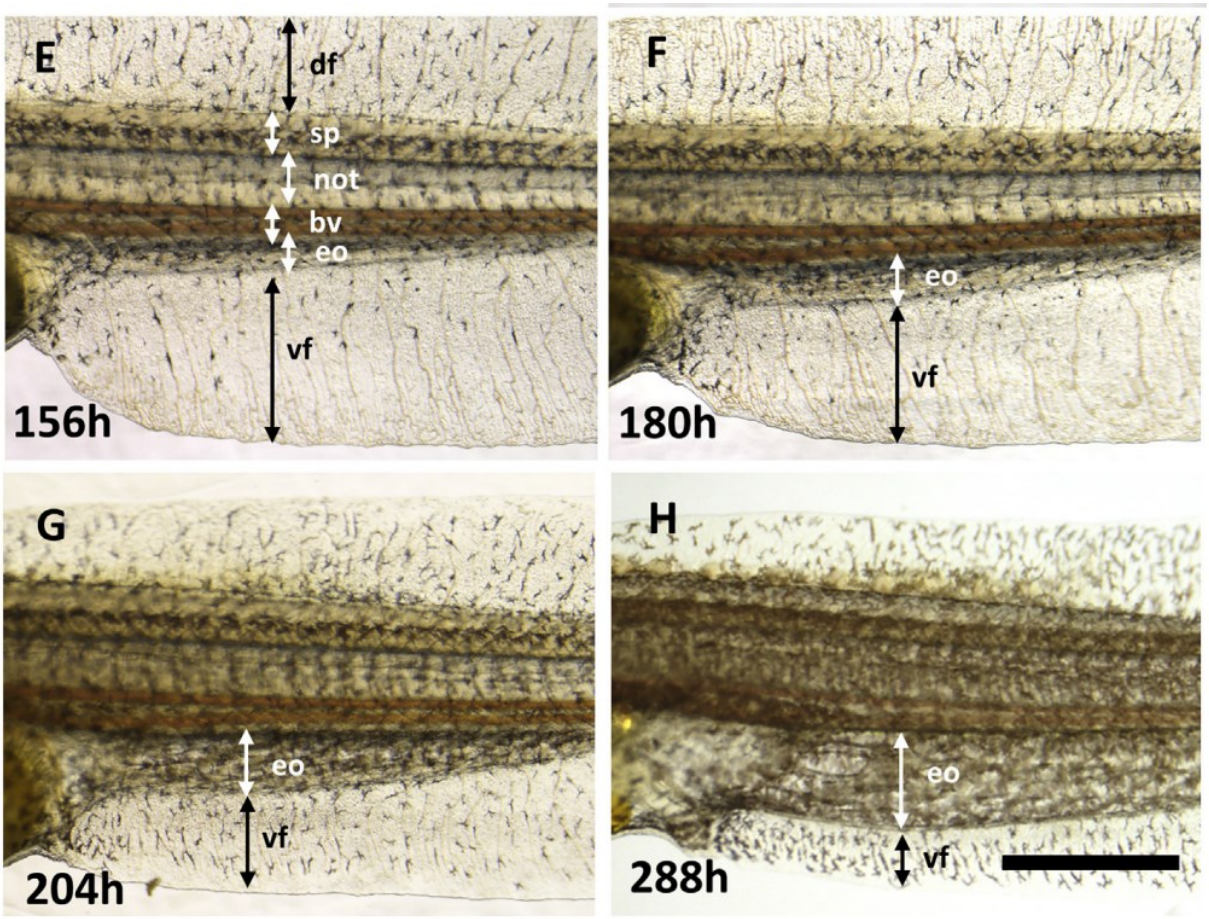

Figure 1. Development of the mEO in the *B. gauderio*. All lateral view with anterior-left. Time is indicated as hours post fertilization: E 156h, F 180h, G 204h and H 288h. df, dorsal embryological fin fold; sp, spinal cord; not, notochord; bv, blood vessel; eo, electric organ; vf, ventral fin. Scale bars = 1 mm. (Adapted from Alshami et al. 2020).

Instrumentation

The RMC Boeckeler PowerTome XL (PT XL) ultramicrotome was used to section fixed embryonic, larval, and adult knifefish specimens. The PT XL is eminently suited for these types of applications, as it is capable of cutting ultrathin sections for transmission electron microscopy (TEM), as well as thicker sections for scanning electron microscopy (SEM) and light microscopy methods. The PT XL is the only fully upgradeable ultramicrotome, with the ability for build-up to the PT PCZ research level system, including touchscreen computer control and HD video collection. The PT XL is capable of accommodating glass knives up to 12mm wide, triangular tungsten carbide knives, and any commercial brand of diamond knife. The PT XL can also be outfitted as a PT 3D, for array tomography and serial sectioning approaches of up to 1 million nanometers in sample depth. The PT XL can also be outfitted for cryo applications or automated section collection and is compatible with medium- and high-throughput add-ons.

Figure 2. The RMC Boeckeler PowerTome XL (PT XL) Ultramicrotome.

Procedure

Tails from the following embryonic and larval stages were collected, fixed, and processed for transmission electron microscopy (TEM): 48 hours post-fertilization (hpf), 60 hpf, 72 hpf 132 hpf; 6.5 days post-fertilization (dpf), 7.5 dpf, 8.5 dpf and 10.5 dpf; in addition to 7 months post-fertilization (mpf) adult samples.

Samples were first fixed in 3% glutaraldehyde with 2% formaldehyde in 0.1 M PIPES (piperazine-N,N′-bis(2-ethanesulfonic acid)) buffer at pH 7.2 for 2 hours at room temperature, then stored at 4° C until use. Samples were then washed (3×, 5 min. each) in 0.1 M PIPES buffer before post-fixation for 1 hour in 1% OsO_4 (osmium tetroxide) reduced with 1.5% potassium ferrocyanide in 0.1 M sodium cacodylate at pH 7.2. After 3×, 5 min. washes in deionized water, samples were dehydrated with an ethanol gradient (1×; 30%, 50%, 70%, 80%, 90%, 95% and then 4× 100% ethanol, for 10 min. each) and subsequently embedded in Spurr's resin for sectioning. The cured resin blocks were manually trimmed with a razor blade prior to ultrathin sectioning with a diamond knife using the RMC Boeckeler PowerTome XL ultramicrotome. 70 nm ultrathin sections were collected on pioloform-coated 100 mesh copper EM grids and contrasted with Reynold's lead citrate for 10 min. The grids were imaged on a JEOL JEM 1400 TEM at 120kV, with images captured on a CCD digital camera.

Results

The authors were able to add to and improve upon both quantitative and qualitative aspects of the developmental staging of knifefish embryos and larvae. This was accomplished primarily through complementary imaging approaches and careful anatomical and cellular analyses. The authors made novel conclusions regarding the origination and translocation of electric organ precursor cells, as well as new insights into the nerve cord and tail regeneration.

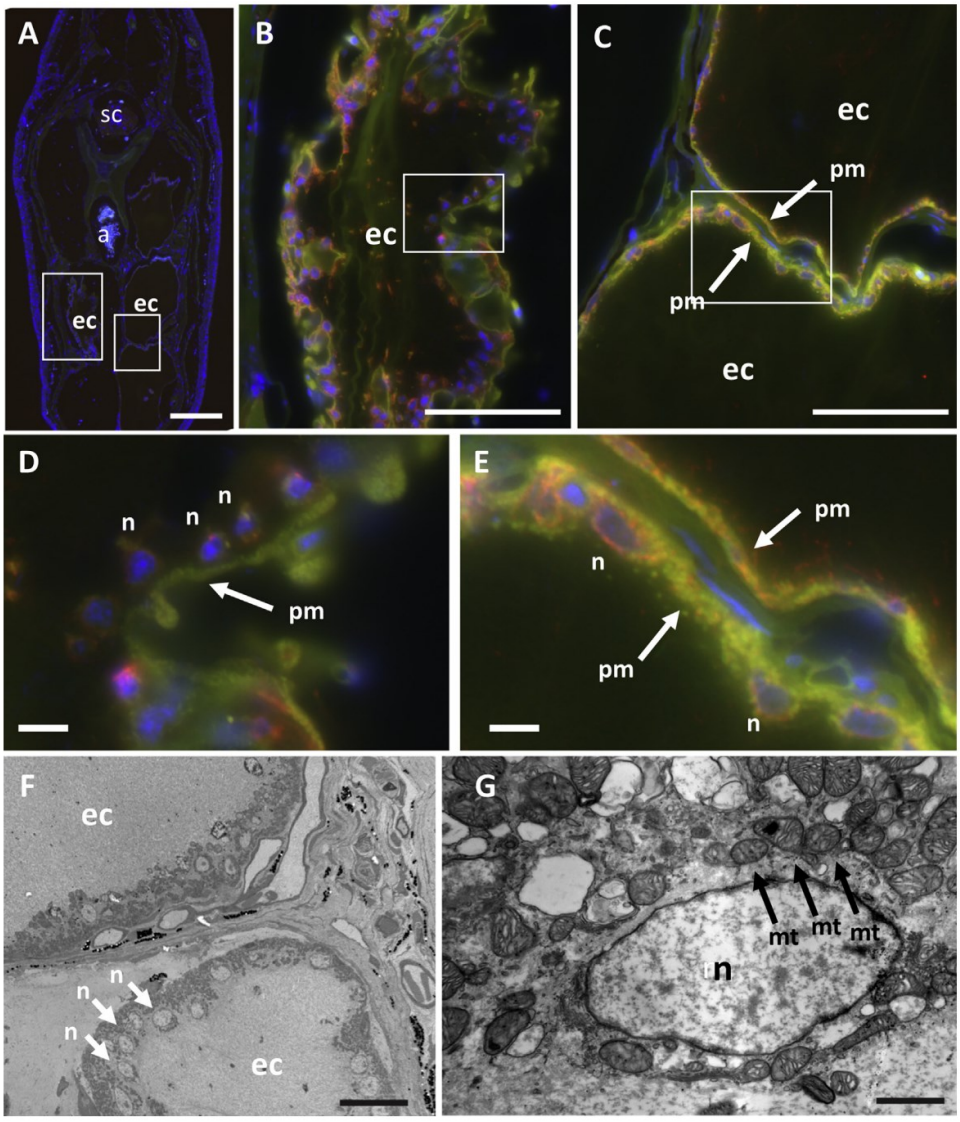

Figure 3. Organelles are accumulated at the periphery of the plasma membrane in the EO. Caudal filament of adult *B. gauderio* was analyzed with immunostaining with alpha-tubulin (red), phalloidin (green), and Hoechst nuclei staining (blue). (A–E), and with TEM (F, G). a, aorta; sc, spinal cord; ec, electrocyte. n, nucleus; mt, mitochondria; pm, plasma membrane. Scale bar = 200 µm in A; 100 µm in B, C; 10 µm in D, E; 20 µm in F; 1 µm in G. (Adapted from Alshami et al. 2020).

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