Expression of anoctamin 1 reveals the presence of interstitial cells of Cajal in the intestine of wild-type and mutant zebrafish

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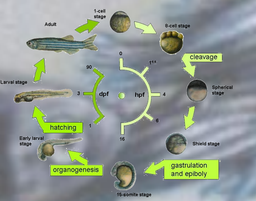
Introduction



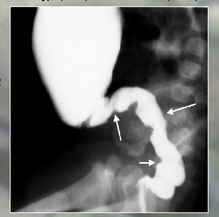
Over the last decade, the zebrafish has emerged as a leading model organism to study vertebrate development and human disease conditions, including functional gastrointestinal (GI) disorders.

Interstitial cells of Cajal (ICC) are specialized cells that generate electrical slow waves initiating GI motility and participate in neurotransmission. In mammals, ICC are predominanlty identified by the expression of the receptor tyrosine kinase, kit. Presence, distribution patterns and expression features of these ICC are known to be affected in various GI motility disorders. Anoctamin 1 (Ano1), a Ca^{2+}-activated Cl^--channel, has recently been shown to be a specific ICC marker in mice, primates and humans. It is shown that Ano1 is essential in slow wave generation and regulates ICC proliferation.

Aim

This study aimed at testing the validity of Ano1 as ICC-marker in the zebrafish intestine and to study its presence and distribution in the intestine of wild-type and mutant zebrafish. The mutant, *lessen*, shows the characteristics of Hirschsprung's disease (HSCR), a congenital disorder characterized by aganglionosis of the distal intestine.

Wild type (A: wt) vs mutant *lessen* (B: *lsn*)



Human colon affected with HSCR

Material and methods

Using immunofluorescence, sections and whole mounts of adult zebrafish intestine as well as isolated intestines of zebrafish embryos and larvae (3 to 6 dpf) were analyzed for the expression of Ano1, along with the panneuronal marker acetylated tubulin (αtub).

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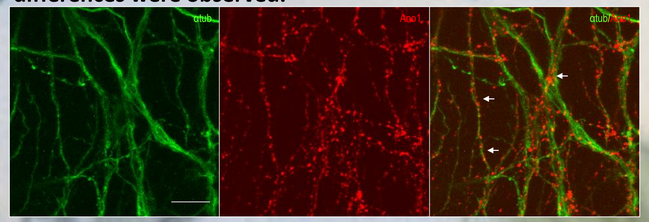
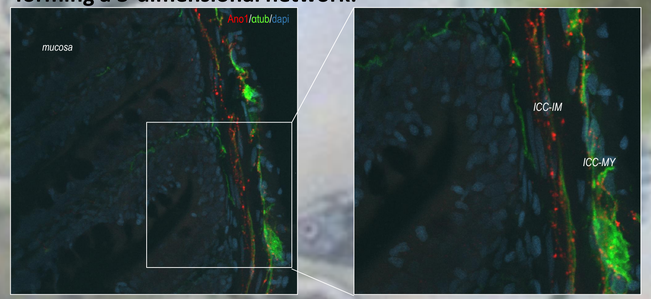
Results

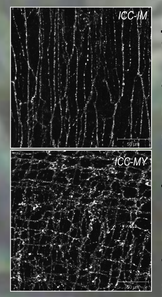
Adult zebrafish

Granular Ano1-immunoreactivity revealed ICC-like cells forming a dense network in the intestinal wall. Colocalizations between αtub immunoreactive nerve fibers and the Ano1 immunoreactive network were observed *(arrows)*, indicating cell-to-cell contact. No regional differences were observed.



Ano1-positive ICC-like cells in two interconnected layers forming a 3-dimensional network:

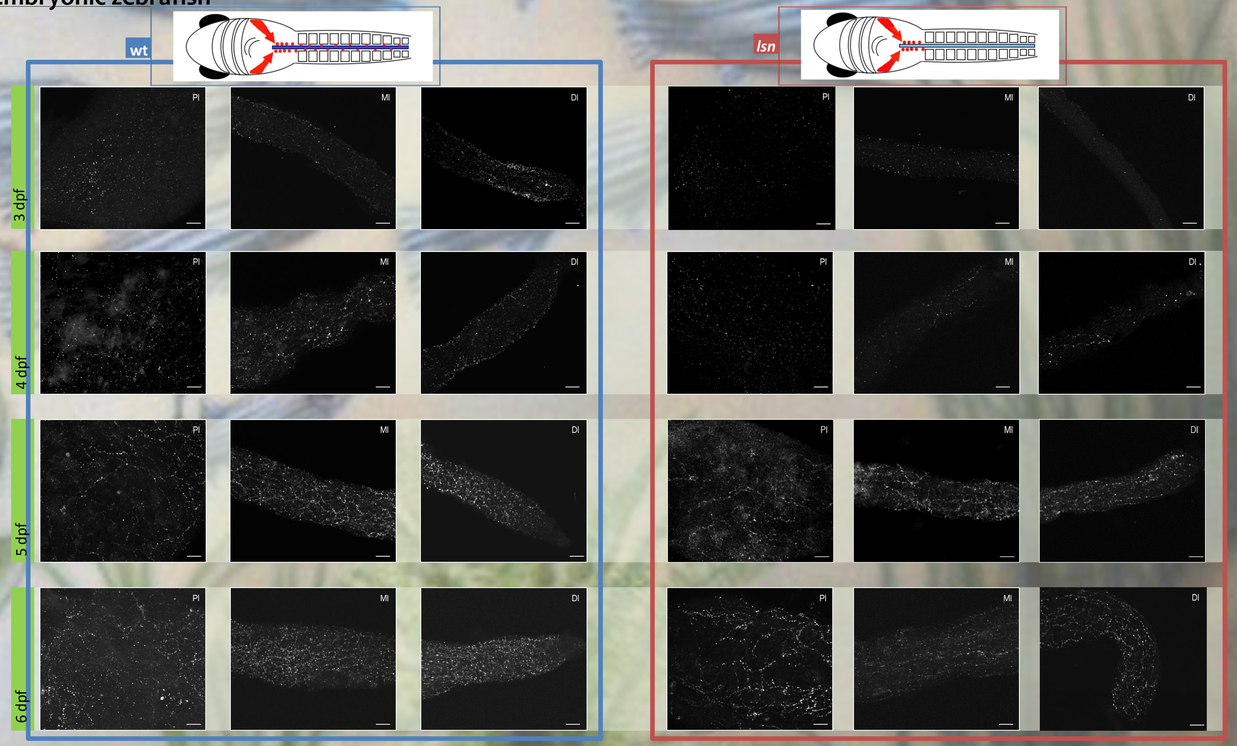




Intramuscular (ICC-IM like):
Within the circular muscle layer, displaying Ano1-positive bipolar cells forming a loose network, oriented along the long axis of the surrounding smooth muscle cells.

Myenteric plexus (ICC-MY like):
In the myenteric plexus between the two muscle layers, intertwined with neuronal fibres, showing multipolar Ano1-immunostained cells.

Embryonic zebrafish



In 3dpf wild-type embryos, few cells expressing faint granular Ano1-immunoreactivity were observed, scattered throughout the intestinal wall. By 5 - 6dpf, a clear 3-dimensional network of cells expressing Ano1 is formed throughout the intestine. In mutant embryos, expression of Ano1 started 1 day later compared to the wild type, and the ICC-like network was less dense, especially in the distal intestine (PI: proximal intestine; MI: middle intestine; DI: distal intestine; bar = 20 µm).

Conclusion: We demonstrated that Ano1, as in mammals, is a selective marker for ICC-like cells in the zebrafish intestine. Ano1-positive ICC-like cells first appear at 3 dpf in the embryonic intestine, indicating that proliferation of ICC-like cells begins at this time point. The expression of Ano1 is delayed in the *lessen* mutant, as observed in human HSCR conditions and mutant mice (*lethal spotted*) showing HSCR characteristics. Furthermore, it is hypothesized that ICC-like cells generate spontaneous contractile activity of the embryonic intestine, because the first appearance of ICC-like cells in the embryonic intestine occurs at the same time that the first spontaneous contractility is observed in the embryonic intestine.

Bars : 20 µm