Regulation of gut peristalsis during development

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Introduction

The digestive system is crucial for food digestion and absorption of nutrition in adults. During feeding, food is transported aborally from the mouth through the esophagus to the stomach, small and large intestines, and each region of the gut exhibits proper movements for their functions. Peristaltic movements in the intestine, recognized as a wavelike propagation of local contraction, is essential for bolus transportation in a spatio-temporally regulated manner. Many gut-related disorders including irritable bowel syndrome and postoperative paralytic ileus are accompanied by peristaltic dysfunctions, resulting in severe digestive problems. Thus, it is important to elucidate the cellular mechanisms by which the gut peristalsis is sustained and regulated.

Recently, it is reported that peristaltic movements are also present in the embryonic gut of several vertebrate species including chickens, even though embryos do not require food intake. Although the physiology of the peristalsis is well studied in the adult body, it remains unexplored how the peristalsis is established during embryogenesis. The embryonic chicken gut serves as an excellent model system, because gut-constituting cells and tissues are easily manipulable experimentally and cells that constitute the chicken gut parallel those of mammals. Chevalier and co-workers reported pioneering studies using chicken embryonic guts, in which they described changes in speed and frequencies of local peristaltic waves during development. These studies focused on the timing of local peristalsis, and the data were acquired using fragments of specific gut regions at specific times. However, the gut is a long organ, where peristalsis occurs widely in a complex manner along the gut axis. Therefore, the spatial information of the peristaltic patterns along the entire gut axis is necessary for understanding the regulation of peristalsis, but such information has been lacking.

Materials and Methods

To obtain the spatial information of the peristaltic patterns along the entire gut axis, the intact gut posterior to the duodenum was dissected from a chicken embryo, and placed into a petri dish with pins to secure the specimen. Since the peristalsis is very sensitive to a mechano-stimulation such as a touch with forceps, the specimen was allowed to rest for 10 min, which was followed by the video recording for another 10 min. The filmed data were processed digitally using ImageJ, and subjected to kymography to assess the peristalsis quantitatively. In the cloaca, changes in the contractions in captured images were converted into intensity values using ImageJ. To investigate how inter-region cross-talks of the gut mature, an optogenetic system using channelrhodopsin2 (ChR2) was developed to experimentally control the gut by a blue-light LED equipped with an optic fiber.

Results and Discussion

In this study, I have produced a "map of peristalsis" along the developing gut posterior to the duodenum, which includes jejunum, ileum, hindgut and caecum. Combining video recordings of non-interrupted gut specimens with kymographic analyses, I examined the distribution pattern of the origins of peristaltic waves (OPWs). From the distribution maps of OPWs, I have found that during the development of the midgut (jejunum and ileum), randomly distributed OPWs become progressively confined to distinct zones along the gut axis, which are largely conserved among different individuals, implying that intrinsic regulations are involved. In addition, the enteric nervous system (ENS) plays a role in the determination where the OPWs arise, and the proper distribution of the OPWs is important for the effective transportation of inter-luminal contents.

Furthermore, I have found that the peristaltic rhythm in the hindgut is tightly coupled with acute contractions of the cloaca (anus-like structure in avians), suggesting a coordination between these tissues. To investigate how inter-region crosstalk of gut

matures, I have developed an optogenetic system to experimentally control the gut motility pattern using channelrhodopsin2 (ChR2). With blue-light LED equipped with an optic fiber, the local light stimulation targeting the hindgut is able to evoke an ectopic peristaltic motility. In addition, the artificial peristaltic waves arriving at the cloaca have induced the acute contractions of the cloaca muscles. These findings show that the acute contraction of cloaca is triggered by the hindgut-derived peristaltic contraction. The peristaltic rhythm in the hindgut is uncoupled with acute contractions of the cloaca at early stages, suggesting that the inter-region crosstalk is progressively established during embryogenesis.

Conclusion

In this study, the spatial information of the peristaltic patterning along the entire embryonic gut was acquired. Furthermore, the optogenetic system that could experimentally control the gut motility was developed. These outcomes provide the fundamental information and technique for investigation of peristalsis. The development of optogenetics combined with quantitative assessments opens a way to understand how the physiological function of gut peristalsis is established during development.