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REVIEW

Emerging role of zinc homeostasis by zinc transporter ZIP7 in intestinal homeostatic self-renewal

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There has been a growing interest in the biological significance of zinc and its regulatory mechanism in the development of tissue and in the maintenance of tissue homeostasis. The intestinal epithelium undergoes a continuous self-renewing process to maintain the intestinal homeostasis. Moreover, dysregulation of this process often causes various intestinal disorders including inflammatory bowel disease, ulcer, and cancer. However, the molecular basis of zinc-dependent regulation of intestinal epithelial cell turnover is not fully understood. In this research highlight, we describe that the zinc transporter ZIP7 (highly expressed in undifferentiated epithelial cells) plays a critical role in the intestinal epithelial self-renewal process by alleviating the ER stress during vigorous proliferative response. Our findings also provide evidence showing that the fine-tuning of intracellular zinc homeostasis by ZIP7 is essential to maintain epithelial homeostasis in the intestine.

Keywords: Zinc transporter; intestinal epithelium; intestinal stem cells; ER stress; transit-amplifying cells

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Zinc homeostasis by zinc transporters

Zinc is an essential trace element required for maintaining the homeostasis of our body [1]. When the level of zinc is decreased in the body, various symptoms associated with deficiency of zinc, such as growth retardation, immunodeficiency, and disordered-homeostasis of intestinal epithelium, are observed [2]. Therefore, a daily dietary intake of 2 g of zinc is required to maintain the functions of the body. The level and distribution of zinc in organs, tissues, and cells of the body are controlled tightly by homeostasis [3, 4]. Zinc homeostasis is maintained by two families of zinc transporters: SLC39A/ZIP and SLC30A/ZnT. Both ZIP and ZnT transporters have been evolutionally conserved from prokaryote to eukaryote and plants and consist of over 20 members [3, 4]. Changes in zinc transporter expression levels and cellular localization take place in response to altered zinc level, thereby facilitating zinc influx or efflux and adjusting

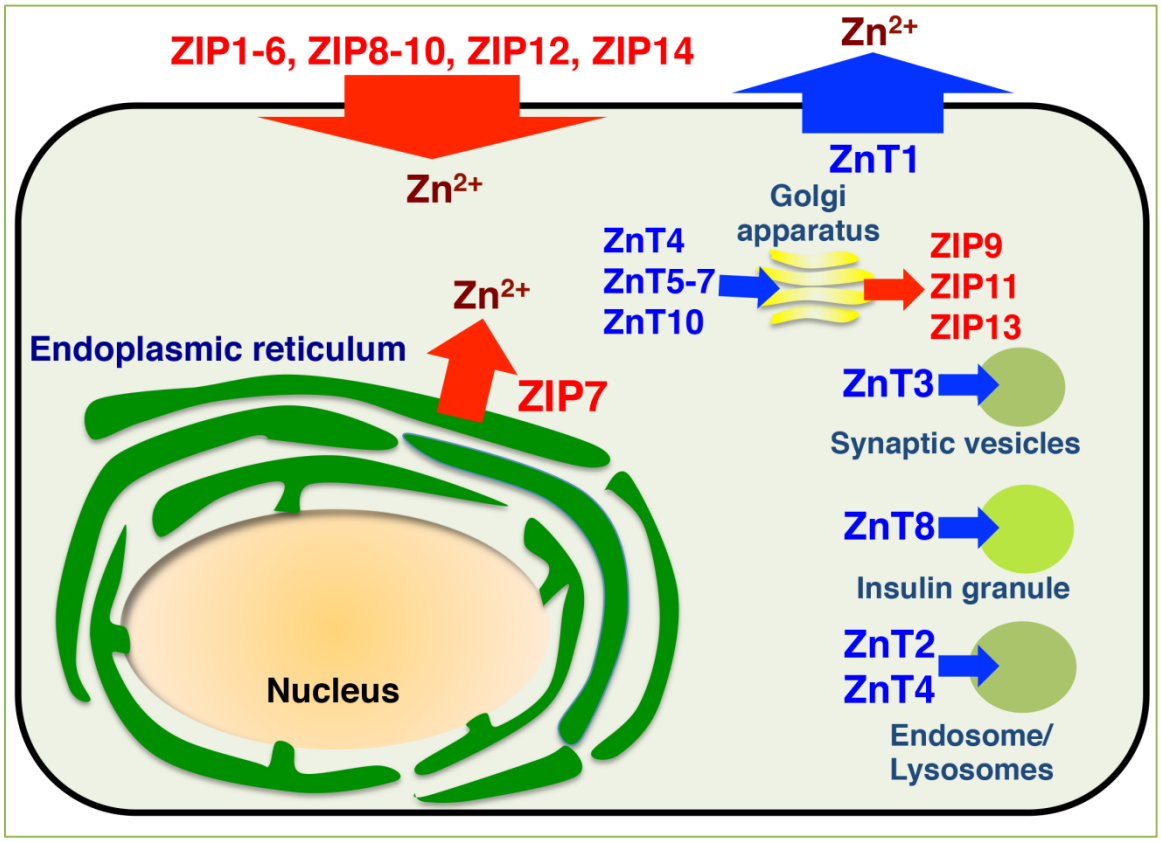

Figure 1. Subcellular localization of zinc transporter. Each zinc transporter has original localization and is distributed throughout the cell membrane. The expression and localization of zinc transporter is altered by various physiological stimuli in response to changes in zinc levels.

the zinc distribution. Above all, physiological stimuli such as cytokines, lipopolysaccharide, hypoxia, and stress also affect the zinc dynamics by changing the expression as well as activity of zinc transporters. Zinc acts as an intracellular second messenger to facilitate certain signaling events [5]. Compelling evidence suggests that zinc signal from zinc transporter controls the organ-specific functions such as connective tissue development [6], systemic growth [7], and immune regulation [8, 9]. Thus, zinc signaling is one of the emerging factors in regulation of physiological and pathological events.

Among SLC39A/ZIP family members, ZIP7 is a unique intracellular zinc transporter that localizes to the endoplasmic reticulum (ER) [10] (Figure 1). The ER is a major intracellular storage of zinc, indicating that ZIP7 plays an important role in regulating cellular zinc homeostasis in the ER. Many studies on fruit fly and plants have reported the biological role of ZIP7. Catsup, the ZIP7 orthologue in *Drosophila*, which functions as a negative regulator of tyrosine hydroxylase activity in the catecholamine biosynthesis [11] and sleep cycle [12] is required for the molting process. The study by Lasswell *et al* [13] showed that IAR1, the ZIP7 orthologue in *Arabidopsis*, is important for the regulation of root morphogenesis. Although in mammals, ZIP7 is ubiquitously expressed in the adult tissues, physiological *in vivo* functions of ZIP7 have remained unknown. In the next part, we have described the roles of zinc homeostasis in intestinal regulation, by mainly focusing on the zinc transporter ZIP7 [14].

Regulation of intestinal epithelium by zinc homeostasis

The surface of the intestine is covered by a monolayer of intestinal epithelium that renews every 3-5 days and is one of the most rapidly self-renewing tissues in adult mammals [15]. Intestinal epithelial lineage cells originate from intestinal stem cells. The intestinal stem cells reside and continuously self-renew and generate daughter cells called transit-amplifying (TA) cells at the intestinal crypt [15]. These TA cells are located in the middle of the crypt and rapidly divide with the limited numbers, after which they differentiate into specialized cells that migrate along the villi and are finally shed into the lumen [15]. The villi are composed of terminally differentiated epithelial cells, namely, absorptive enterocyte and secretory cell lineages including goblet cells, tuft cells, and enteroendocrine cells. In contrast, Paneth cells, another secretory cell lineage, reside at

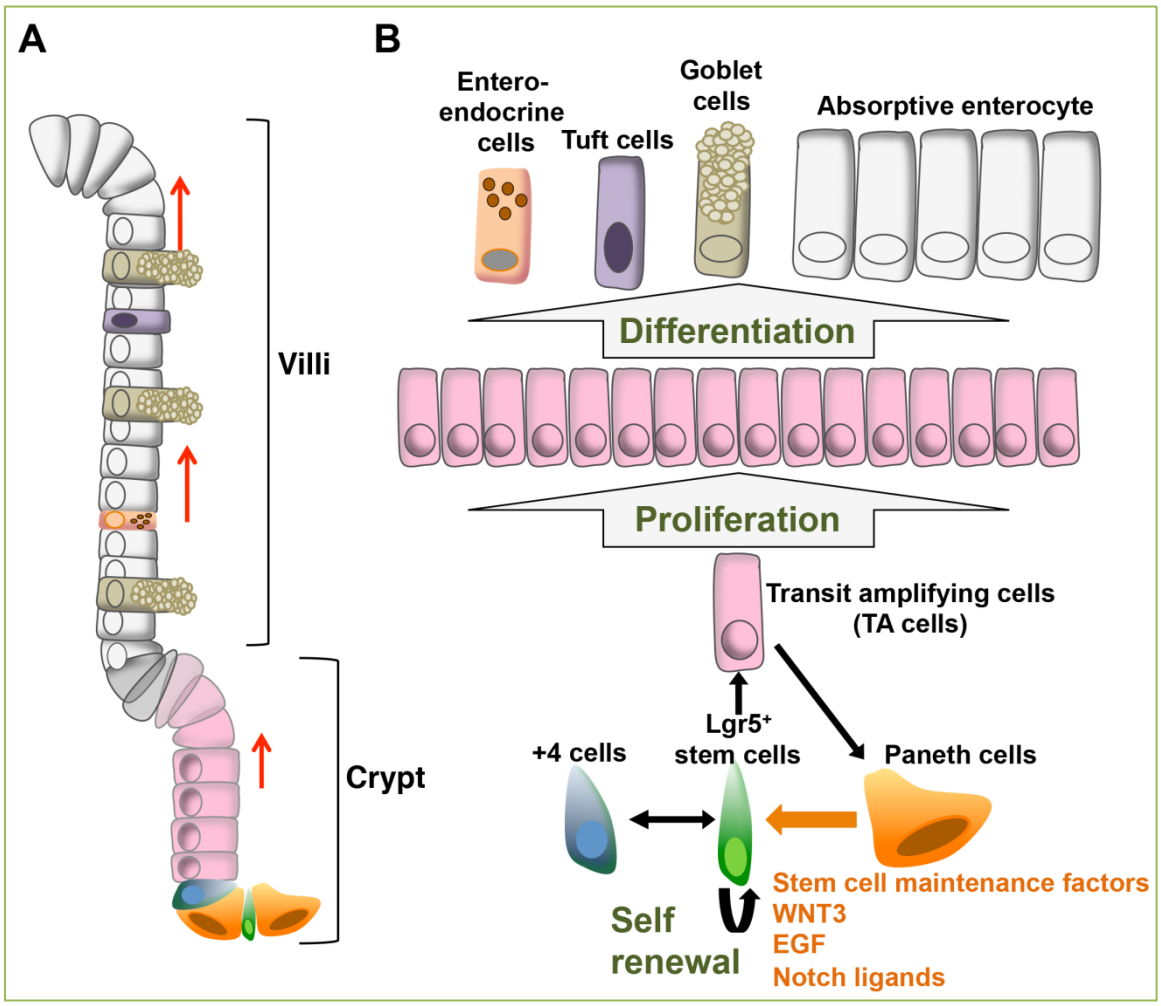

Figure 2. Structure of small intestine and the intestinal epithelial self-renewal process. (A) The structure ofthe small intestine consisting of villi-crypt units is shown. At the crypt base, Lgr5$^+$ CBC cells are located and interspaced between Paneth cells. +4 cells are located just above Paneth cells. Proliferating TA cells occupy the middle region of crypts and migrate along the villi when they begin to differentiate. (B) The hierarchy of the intestinal epithelial turnover is shown. Intestinal stem cells generate proliferative TA cells that in turn produce large numbers of differentiated cells. TA cells also produce Paneth cells that are located at the crypt base.

the crypt base to provide stem-cell-maintenance factors such as Wnt, Notch ligands, and EGF [16]. Thus, the intestinal crypts are highly proliferative compartments and are responsible for providing vigorous epithelial cell turnover and maintenance of the stem cells (Figure 2). We found that ZIP7 was highly expressed in this region, and therefore we investigated the roles of ZIP7 in the maintenance of intestinal epithelial homeostasis, as described below [14].

ZIP7 is expressed in the intestinal crypt where the intestinal self-renewal process takes place

We found that ZIP7 was highly expressed in the crypt epithelium than in the villus epithelium [14]. Intestinal crypts are the region where intestinal stem cells are self-renewed and generate TA cells. We observed that both rapid cycling TA cells and terminally differentiated Paneth cells express ZIP7. In the intestinal crypt, two types of intestinal stem cells have been identified. One is the crypt base columnar (CBC) cells that are interspersed between Paneth cells [17] and the other is +4 cells that occupy the fourth position from the crypt base, located above the Paneth cell compartment [17]. The +4 cells are thought to be quiescent cells, whereas CBC cells are actively cycling and are marked by *Lgr5* or *Olfm4* [17]. We also found that ZIP7 is expressed in active cycling *Olfm4*$^+$ stem cells indicating that ZIP7 may have an important role in the intestinal self-renewal process [14].

Loss of ZIP7 causes impaired self-renewal of intestinal epithelium

These findings led us to speculate on the contribution of ZIP7 in the renewing process of intestinal epithelium. Therefore, we generated a tamoxifen-induced intestinal

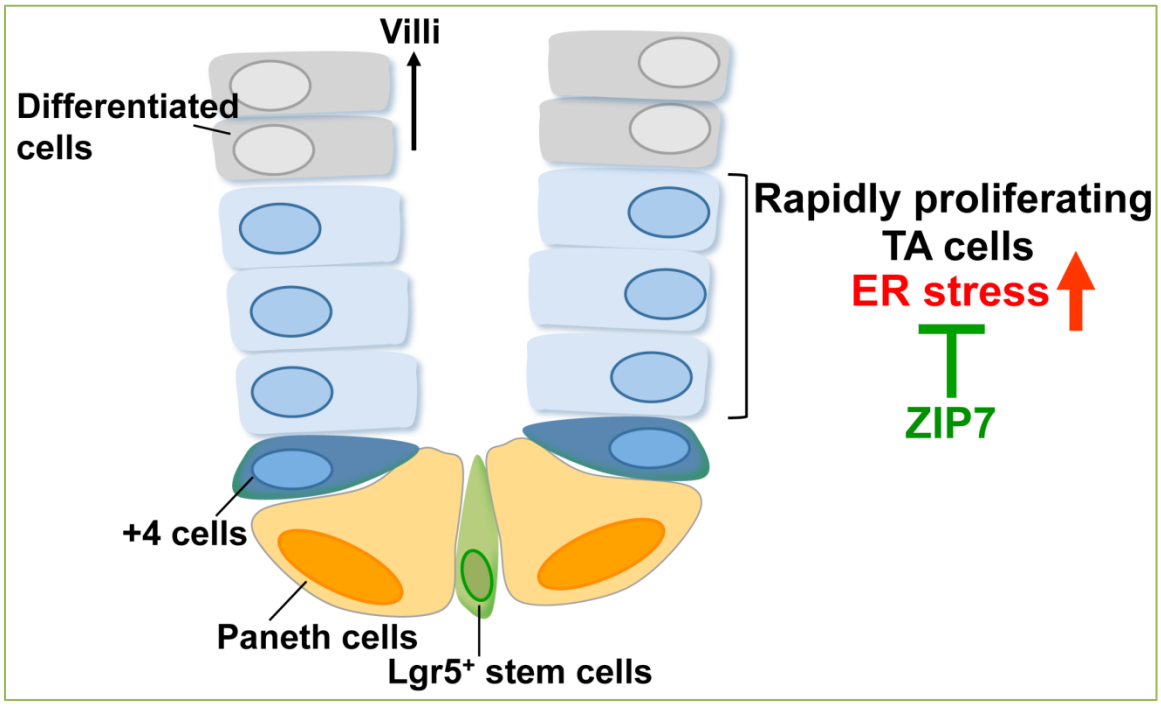

Figure 3. Model showing the role of ZIP7 in the intestinal epithelial self-renewal process. Intestinal stem cells located at the bottom of the crypt undergo the self-renewal process and generate proliferative TA cells. These cells proliferate vigorously, producing large numbers of differentiated cells. During this proliferation, ZIP7 is induced, and this resolves the upregulated ER stress, thereby ensuring proliferative responses in intestinal crypts.

epithelium-specific *Zip7* knockout mice (*Zip7*$^{fl/fl}$;*Vil-Cre*^{ERT2} mice, referred to hereafter *Zip7*$^{\Delta IEC}$ mice) and evaluated the impact of ZIP7 loss on epithelial homeostasis and regeneration. In these mice, treatment with tamoxifen induced intestinal epithelium-specific deletion of *Zip7*. After the tamoxifen treatment, *Zip7*$^{\Delta IEC}$ mice died within a week. The induction of ZIP7 deletion rapidly caused impaired villus-crypt structure and loss of Ki67^{+} TA cells within a few days after the initial treatment with tamoxifen. Notably, the loss of ZIP7 led to a substantial apoptosis of TA cells and degeneration of Paneth cells. *Olfm4*$^{+}$ intestinal stem cells were completely eliminated on deletion of ZIP7. Thus, our findings provide the first evidence showing that ZIP7 is essential for epithelial proliferation and stem cell maintenance during normal homeostasis of the small intestine[14]. *Lgr5*$^{+}$ (*Olfm4*$^{+}$) stem cells are essential for regeneration after cells are damaged due to irradiation[18]. We determined the role of ZIP7 in cycling *Lgr5*$^{+}$ stem cells using *Lgr5*$^{+}$ stem cell-specific *Zip7*-deficient mice (*Zip7*$^{fl/fl}$; *Lgr5*^{EGFP}-*IRES*-*Cre*^{ERT2}) and demonstrated that *Lgr5*$^{+}$ stem cell-specific *Zip7*-deficient mice were highly sensitive to irradiation and exhibited lower lethal dose of radiation. Therefore, our findings suggest that ZIP7 in *Lgr5*$^{+}$ stem cells is essential for intestinal regeneration after damage due to radiation[14].

Loss of ZIP7 elevates ER stress

Intestinal stem cells give rise to rapidly cycling TA cells that are responsible for building tissue mass [19]; therefore, studies on apoptosis of TA cells caused by loss of ZIP7 was carried out. We found that the deficiency of ZIP7 greatly enhanced ER stress that activated apoptotic signaling in TA cells, as well as in intestinal stem cells[14]. Ectopic expression of ZIP7 protein ameliorated the increased ER stress in the *Zip7*-deficient MEF cells. In contrast, exogenous zinc supplementation did not have any effect on the increased ER stress and could not restore the self-renewal capacity in *Zip7*$^{\Delta IEC}$ crypt in an organoid culture system and in *Zip7*-deficient MEF cells[14]. As ZIP family can import zinc from an extracellular region or intracellular compartment to cytosol, the ER-localized zinc transporter, ZIP7, most likely contributed to the regulation of zinc influx from the ER to cytosol. Therefore, the fine-tuning of intracellular zinc homeostasis by ZIP7 is indispensable for ensuring the self-renewal process of intestinal epithelium and regulation of the ER function[14] (Figure 3).

Conclusions

Growing evidence has shown that tight regulation of zinc distribution and flux by zinc transporter contribute to the regulation of tissue function; however, disturbances in zinc

homeostasis result in the dysregulation of various tissue functions and facilitation of the pathogenesis processes. It has been reported that in the intestine, ZIP4 and ZnT2 contribute to the maintenance of Paneth cell and zinc storage in their granules [20, 21]. Our study provides the first evidence of the essential role of zinc transporter in the homeostatic intestinal epithelial self-renewal and regeneration events after injury. Our studies presenting a novel mechanism of maintenance of intestinal homeostasis by ER-localized zinc transporter, ZIP7 (Figure 3), may extend our knowledge of molecular mechanisms underlying intestinal epithelium undergoing continuous self-renewing processes. Although ER serves as intracellular zinc storage, the role of zinc in ER function is not fully understood. Furthermore, given that various cells proliferate vigorously under certain physiological and pathological conditions, our study provides an insight into the mechanisms by which cells adapt to undergo a rapid proliferation through fine-tuning ER function by ZIP7-mediated zinc signal. ZIP7 is expressed in many tissues; therefore further studies should be done to explore the role of ZIP7 in other tissues and also mechanisms by which ER zinc signals contribute in maintaining tissue homeostasis and pathogenesis of various diseases.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Abbreviations

CBC: crypt base columnar; EGF: epidermal growth factor; ER: endoplasmic reticulum; MEF: mouse embryonic fibroblast; TA: transit amplifying; UPR: unfolded protein response.

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