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REGULATION OF *DROSOPHILA* INTESTINAL STEM CELL MAINTENANCE AND PROLIFERATION

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Abstract. To maintain gut homeostasis intestinal stem cells (ISCs) constantly replace damaged ones. This process is conservative from *Drosophila* to human. Proliferation and differentiation of ISCs in adult *Drosophila* midgut are regulated by growth factors which are secreted in the surrounding cells collectively forming ISCs niche. Here I discuss an interaction between ISCs with its niche through conservative signaling pathways. Several evidences on significance of cooperation between multiple signaling pathways including Notch, Wingless, JAK/STAT, EGFR, Hippo, and insulin signaling for regulation of stem cell maintenance and activity are provided. Further investigation in this area will allow us to understand how proper regulation of ISCs maintenance and differentiation can assist to ensure intestinal integrity.

Keywords: intestinal stem cells, *Drosophila*, niche, signaling pathway, midgut.

Abbreviations: ISCs, intestinal stem cells; EBs, enteroblasts; ECs, enterocytes; EEs, enteroendorcine cells.

1. INTRODUCTION

The digestive tract of *Drosophila melanogaster* plays a key role in digestion, absorbtion, transit, and excretion. *Drosophila* gut is one of the biggest organs in its body. It consists of foregut, midgut and hindgut. The midgut is composed of an epithelium covered by visceral muscle. The epithelium consists of intestinal stem cells (ISCs), enterocytes (ECs), and enteroendocrines (EEs). All these cells form the niche which controls the ISCs proliferation and differentiation of enteroblasts (EBs). Undergoing asymmetric division, ISC can produce two types of cells. One such type, stem cells, to keep the amount of ISCs constant and the second counterpart, enteroblasts, which further differentiate into enterocyte or enteroendocrine cell [22].

There are several signaling pathways like Notch, Wingless (Wg)/Wnt, and JAK-STAT pathways, which regulate maintenance, proliferation, and differentiation of ISCs [5, 13, 19]. Understanding of molecular mechanisms will allow us to uncover developmental origin of adult stem cells, the role of their niches and how the destiny of stem cells and their progenies are regulated.

In this review, I describe how intestinal stem cell niche creates an environment, which regulates self-renewal and differentiation of ISCs.

2. INTESTINAL STEM CELL NICHES

The adult *Drosophila* midgut has many similarities to the mammalian intestine and is well characterized. It is an attractive and useful model for investigation the ways of regulation stem cells maintenance, differentiation, and proliferation [7]. The *Drosophila* midgut consists of ISCs which are located on the basement membrane of the epithelium (Fig. 1). ISCs undergo frequent asymetric divisions. In average, midgut cells are renewed approximately four times during the eight week lifespan of the adult female fly [13]. After division, one dauther cell maintain its stemness and the other one become enteroblast, which subsequently differentiates into enterocyte or enteroendorcine. Approximately ninety percents of the EBs will become EC cells, and the rest will become EE cells [25]. These four cell types are characterized by specific array of differently expressed genes.

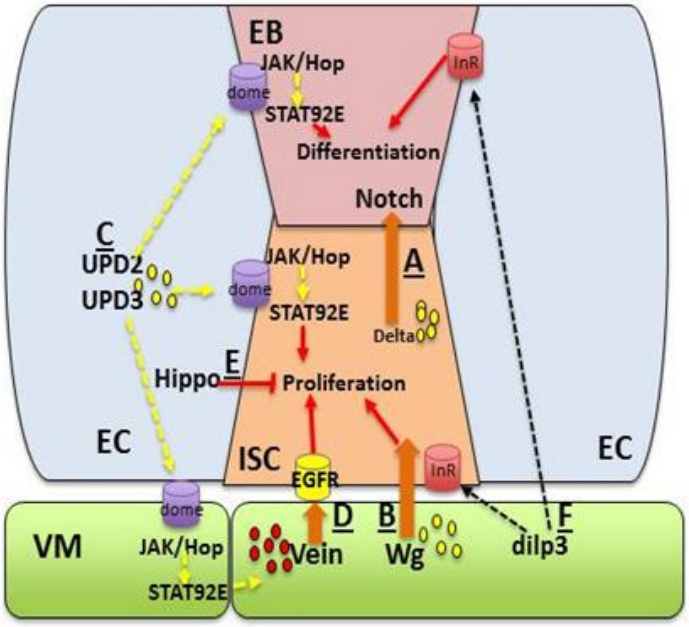

Fig. 1. Input signals from surrounding niche cells that regulate ISCs maintaining, differentiation and proliferation. The diagram represents the genetic interactions between ISCs and the cells of their niche. VM – visceral muscle; ISC – intestinal stem cell; EB – enteroblast; EC – enterocyte; Wg – Wingless; Upds – unpaireds; JAK – receptor-associated Janus Kinase; Hop – Hopscotch; STAT – Signal Transducer and Activator of Transcription; EGFR – Epidermal growth factor receptor; Dome – Domeless; InR – insulin receptor; dilp – Drosophila insulin like peptide.

ISCs are diploid, have a small nucleus and express ligand Delta, which is specific for the receptor of Notch-signaling pathways. EBs are diploid with a small nucleus and express the transcriptional reporter for Notch – Supressor of hairless (SuH) [31]. ECs are polyploid with a large nucleus and express transcriptional factor Pdm1. EEs are diploid cells with a small nucleus and express transcriptional factor Prospero.

ISCs contact with each other via visceral muscle integrins. The muscle express several potential niche factors that are capable to promote ISCs dividing, including wingless (Wg), vein (an Egfr ligand), and dilp3 (an insulin-like peptide) [19, 35]. That is why visceral muscle serves as the stem cell niche. On the other hand, ECs and EEs produce growth factors that regulate stemness and differentiation. These factors are important during regeneration after damage. During midgut development ISCs proliferate intensively and form clusters. Some cells in these clusters become peripheral cells and serve as a transient niche for the progenitors [21]. Cells in this niche produce Dpp (Decapentaplegic) that inhibit differentiation and epidermal growth factor receptor (EGFR) ligands.

3. NOTCH SIGNALING

There is a variety of evidences on Notch as a major regulator of ISCs self-renewal. Notch signaling is evolutionary conserved mechanism important for development. ISCs control differentiation of daughter cell by modulating Notch signaling [25], and Notch is specifically activated in EBs (Fig. 1) [36]. The Notch proteins (single-pass receptor) are activated by Delta ligand. They are transported to the plasma membrane and induce dissociation of Notch intracellular domain (NICD) from the plasma membrane. In the absence of NICD, the DNA-binding protein CLS associates with ubiquitous corepressor (Co-R) proteins and histone deacetylases (HDACs). These factors inhibit the expression of Notch target genes [12]. NICD binds to the CSL and in this way triggers the splitting of transcriptional repressors. Binding of NICD-CLS complex to the components of activation complex MAML1 and HDACs facilitates transcription activation of Notch target genes (Fig. 2) [12]. Numerous of findings indicate that proliferating cells and ECs require the activity of Notch. The downstream transcriptional repressor Hairless prevents transcription of Notch target genes and is sufficient for ISCs self-renewal [3].

Notch signaling has dual role: it controls balance between stem cells and their differentiating progeny and determines the type of progeny [32]. Notch pathway activates Hes-1, the bHLH transcription factor, which is expressed by ECs. The activity of Notch signaling is low in EEs and ECs, which express Math1 and Neurogenin 3, the bHLH proteins [17, 29]. Interestingly, these proteins belong to the same bHLH family and this may show common evolutionary origin of ECs and EEs [10]. Notch is a central signal mechanism for stemness maintaining.

4. WINGLESS (WG)/WNT SIGNALING

Investigation of Lin et al. [19] demonstrated the role of Wnt/Wg signaling in gut homeostesis and the role of ISC niche in the communication between intestinal epithelium and neighboring tissues (Fig. 1). Visceral muscle were found to produce a Wnt ligand, wingless (wg) (Fig. 2) which is very important for ISC maintenance [35]. It was observed that wg is expressed in the visceral muscle and Wg protein is located between visceral muscle and basement membrane [16]. According to this observation, visceral muscle secret Wg through basement membrane. Loss of wg significantly reduces the ISC number [19]. The proliferating effect of Wnt/Wg signaling is mild and the cooperation of Wnt/Wg with EGFR and JAK/STAT regulates a balance between ISC proliferation and maintenance [35].

Wingless activates proliferation that is why loss of this signal results in a loss of dividing cells, and also causes apoptotic cell death [33]. But ISCs proliferation induced by Wnt signaling is much weaker than induced by JAK/STAT or EGFR [14, 22]. Hence one can suggest that other signaling pathways play more important role in ISCs proliferation.

5. JAK/STAT

The JAK/STAT system consists of a receptor, Janus kinase (JAK), and signal transducer and activator of transcription (STAT) [1]. The JAK/STAT signaling pathway is highly conserved from flies to mammals, and plays essential roles during development. It also serves as a regulator of stem cells and their niches. *Drosophila* possesses relatively not complex JAK/STAT pathway. Signaling ligands for this pathway Upd2 and 3 (Unpaireds) both contribute to ISC mitosis, and both of them trigger synthesis of the antimicrobial peptide drosomycin-3. Upds are sufficient to activate JAK/STAT pathway activity in ISCs [4].

A ligand binding to the receptor Dome (Domeless) activates the associated JAK Hopscotch (Hop), which further recruits and phosphorylates the transcription factor STAT92E and finally these events

result in signaling triggering (Fig. 1) [4]. Dimerization of STAT92E allows its translocation into the nucleus where it activates expression of target genes (Fig. 2).

During normal tissue homeostasis in the *Drosophila* midgut, JAK/STAT signaling, does not act on itself, but is a part of a greater regulatory network. It coordinates collective operation of EGFR and Wg signaling pathways. This coordination is necessary for ISC maintenance, and promotes differentiation of EBs. It is suggested, that stresses or infections upregulate JAK/STAT signaling. In these conditions, the signals back from EC and EB to ISC and promote ISC proliferation [4].

6. EGFR

The study of Buchon et al. [6] revealed that a canonical EGFR pathway is required in ISCs and promotes their proliferation. EGFR pathway is activated in the adult intestine by three EGFs, namely Vein (Vn), Spitz (Spi), and Keren (Krn) [6, 35]. All three ligands have overlapping functions in activating of EGFR signaling [13]. Vn is expressed in visceral muscle (Fig. 1) whether Spi and Krn are expressed in the midgut epithelium [6, 35]. It was shown that flies with reduced EGFR activity in ISCs fail to repair their intestine after infection [6]. While Vn is partially regulated in visceral muscle by JAK/STAT, it was hypothesized that visceral muscles serve as a connective tissue between damaged ECs and ISCs [6].

EGFR and JAK/STAT are activated in response to regenerating inflammatory signaling. The interplay between these pathways is realized at the level of ligand induction. Interesting, the inhibition of EGFR completely blocs proliferation, that triggered by Upd3 or Dome in ISCs, while JAK/STAT inhibition only partially depresses proliferation which was induced by EGFR [6]. This fact confirms idea that EGFR plays more important role in ISCs proliferation. Besides EGFR and JAK/STAT control ISC proliferation they have very distinct functions while JAK/STAT controls EBs differentiation, EGFR is required for proper gut morphogenesis [6, 14]. It was shown that both EGFR and JAK/STAT are induced by lack of Hippo signaling in the ECs [28].

7. HIPPO SIGNALING

Hippo signaling was first discovered in *D. melanogaster* as a key regulator of body size. The *Drosophila* Hippo signaling pathway is highly conserved with all main components which have its homologues in mammals [27]. Signal transduction between the mammalian Hippo components is also analogous to that in flies. Hippo signaling negatively regulates stem cell proliferation through limiting cytokine and mitogen biosynthesis that activate JAK/STAT and EGFR pathways (Fig. 1) [28, 30]. It was shown that Hippo signaling is a damage sensor in the intestinal epithelium and other signaling pathways and it plays important role in the control of gut homeostasis.

The Hippo pathway includes Expanded (Ex), the serine-threonine kinases, Hippo (Hpo) and Warts (Wts), scaffolding protein Salvador (Sav), the Mats (Mob and tumor suppressor) and the transcriptional enhancer Yorkie (Yki) [11]. This pathway controls expression of cyclin E and DIAP by modulation Yki activity. There are several conserved components in mammals including *sav* (*hWW45*) and *mats* (*MATS1*). MST1 and MST2 (kinases) and their regulatory protein WW45. For activation, WW45 forms a core complex of the signaling pathway. RASSF family proteins activate MST1/2 by binding and recruiting it to the cell membrane [24]. Activated MST1/2 phosphorylates LATS1 and LATS2 (tumor suppressor homolog kinases), which are regulated by MOBKL1A/B. MST1/2 phosphorylates MOBKL1A/B promoting binding it to the LATS1/2 and they form a complex MOB1-LATS1/2 [26]. Lats1/2 phosphorylates YAP/TAZ, which interacts with 14-3-3 protein and modulates the cytoplasmic retention and trigger protein degradation.

It was demonstrated that Hippo signaling inhibits ISCs proliferation in the adult midgut and was described on-cell-autonomous mechanism of negative regulation of ISCs proliferation. This regulation

is realized through the restriction of cytokines and mitogen production, which can activate JAK/STAT and EGFR cascades [28].

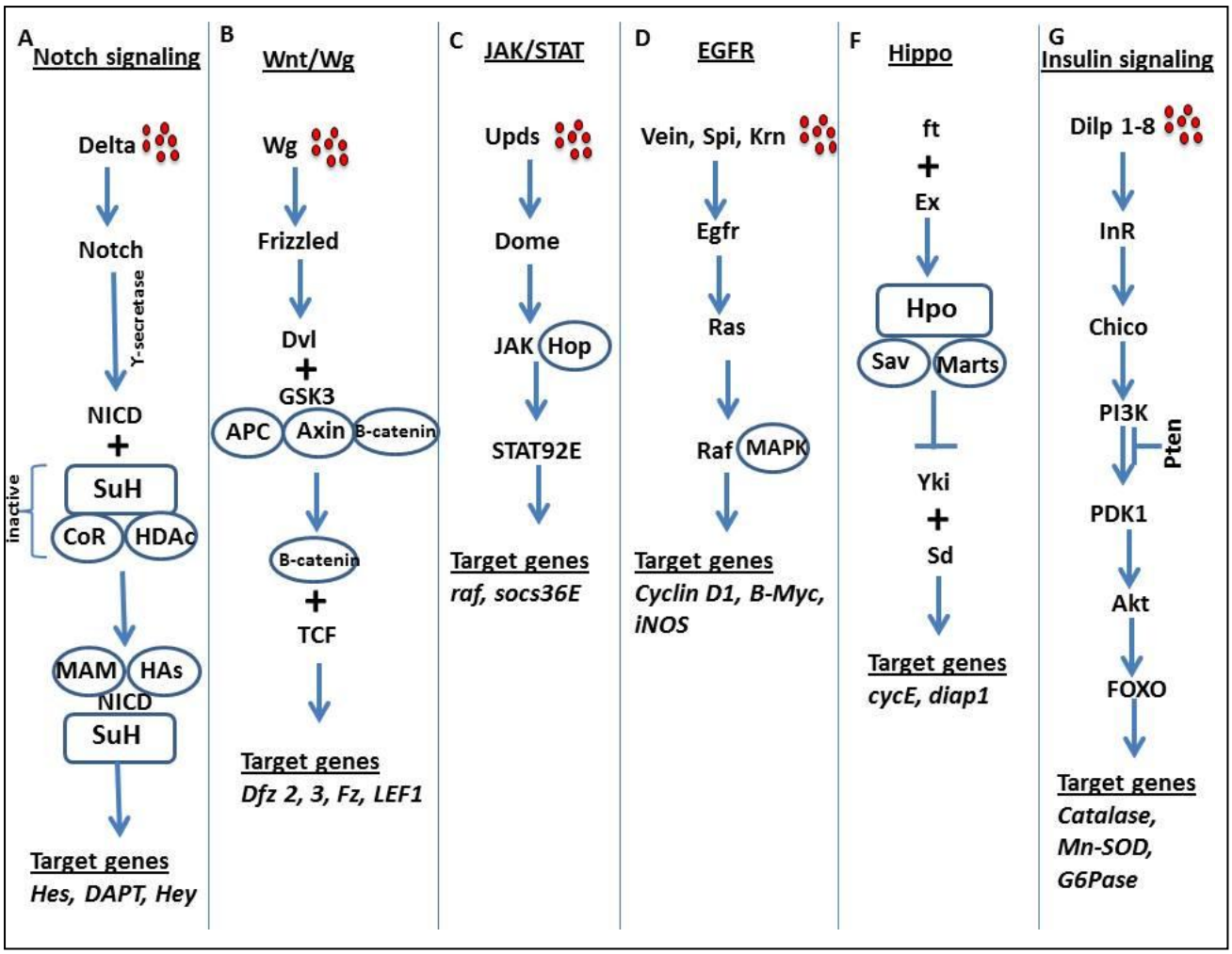

Fig. 2. Signaling pathways involved in interactions between ISCs and the niche cells.
(A) Ligand Delta activates Notch proteins in the EBs. Dissociation of Notch intracellular domain (NICD) from cell membrane is catalyzed be gamma-secretase. Than NICD forms a complex with Suppressor of Hairless (Su(H)) protein to replace a histone deacetylase (HDAc)/corepressor (CoR) from the Su(H). Active complex contains Mastermind (MAM) protein and histone acetyltransferases (HAc) which are recruited to the NICD/Su(H).
(B) Visceral muscle produces Wingless which activates the frizzled receptor (Fzd). This causes an activation of Dishevelled (Dvl), which binds to glycogen synthase kinase-3 (GSK-3) and inhibits it. As a result, ß-catenin dissociates from a complex which is composed of scaffold proteins Axin and APC (Adenomatous polyposis coli). β-catenin triggers the transcription of target genes.
(C) Unpaired 2 and 3 (Upd 2 and 3) activate a receptor-associated Janus Kinase (JAK) termed Hopscotch (Hop) which in turn triggers a Signal Transducer and Activator of Transcription (STAT).
(D) Epidermal growth factor receptor (EGFR) is activated by ligands Vein, Spitz and Kern which stimulate EGFR and activate RAS-RAF-MAPK signaling.
(E) Fat (ft) interacts with Expanded (Ex) and activates the core kinase cascade which includes kinase Hippo (Hpo), adaptor proteins Marts and Salvador (Sav). The main function is to inhibit phosphorylation of transcription co-activator Yorkie (Yki) causing its translocation to the nucleus and binds to transcription activator Scalloped (Sd).
(F) Dilp 3 acts directly through insulin receptor (InR) to activate Protein kinase B (Akt), which phosphorylates and inactivates FOXO.

8. The Role of Diet in ISC Proliferation

It was found that starvation reduces insulin signaling allowing gut to decrease in size during starvation [23]. The insulin signaling positively regulates ISCs proliferation and differentiation during aging and regeneration [2, 8]. Nutrient deprivation leads to inhibition of ISCs proliferation in

Drosophila [8]. Protein deprivation and reduced insulin signaling lead to increased number of lower ploidy enterocyte daughter cells per midgut. These observations indicate that endoreduplication in the midgut is regulated by nutrition [8].

Drosophila intestine expresses two insulin-like peptides Dilp3 and Dilp7 [9, 34]. Dilp7 is expressed in the neurons and in this way regulates intestinal physiology [9]. Dilp3 is expressed in the midgut and foregut muscles (Fig. 1) [34] and acts directly through *Drosophila* insulin receptor (InR) to induce proliferation and midgut growth via asymmetric and symmetric division (Fig. 2) [23]. Interestingly, blocking of brain neurons which produce Dilps leads to inhibition of bleomycin-induced midgut regeneration [2].

9. CONCLUSIONS

This review described the main signaling pathways that control maintenance and differentiation of *Drosophila* ISCs. Several conserved signaling pathways including Notch, Wnt/Wg, JAK/STAT, EGFR, Hippo, and insulin signaling are known to be involved here. Operation of these pathways depends on ISC niche and the ligands they produce. The intestinal regeneration is not controlled by individual signaling pathways, but mainly complex interplay between them. Regulatory signals exchange between epithelium and surrounding tissues to control gut homeostasis. Moreover, tissue damage induces ISC proliferation to replace damaged cells. Under these conditions JAK/STAT and insulin signaling act as important mediators of ISCs proliferation [2, 6, 13]. Little is known about the mechanisms of ISC decision between self-renewal and initiation of differentiation. It worth to underline high conservative level of all these signaling pathways. That is why fruit fly *Drosophila* plays very important role in our understanding many conserved characteristics of stem cells in animals.

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Функціонування стовбурових клітини кишківника (СКК) необхідне для підтримання цілісності шлунково-кишкового тракту за нормальних умов та при пошкодженнях. Задіяні регуляторні процеси характеризуються значною подібністю у *Drosophila* і людини. Проліферація та диференціація СКК кишківника дорослої дрозофіли регулюються факторами росту, що секретуються навколишніми клітинами, які формують нішу СКК. В даному огляді обговорюються механізми регуляції функціонування СКК клітинами ніші через консервативні сигнальні шляхи. Також представлені докази значимості взаємодії сигнальних шляхів Notch, Wingless, JAK/STAT, EGFR, Hippo та інсулінового шляху для забезпечення системності і функціонування СКК. Подальші дослідження в даній області допоможуть зрозуміти як належна регуляція диференціації СКК може сприяти забезпеченню цілісності кишківника.

Ключові слова: стовбурові клітини кишківника, *Drosophila*, ніша, сигнальний шлях, кишківник.