Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Insect Science

miRNA functions in stem cells and their niches: lessons from the *Drosophila* ovary

Halyna R Shcherbata[1,2]

From the very beginning of the miRNA era, *Drosophila* has served as an excellent model for explanation of miRNA biogenesis. Now *Drosophila* continues to be used in numerous studies aiming to decipher biological roles of individual miRNAs in a living organism. MiRNAs have emerged as an important regulatory class that adjusts gene expression in response to stress; therefore, it is particularly important to elucidate miRNA-based regulatory networks that appear in response to fluctuations in intrinsic and extrinsic environments. This review explores the major advances in understanding condition-dependent roles of miRNAs in adult stem cell biology using the *Drosophila* ovarian germline stem cell niche community as a model system.

Addresses
[1] Max Planck Research Group of Gene Expression and Signaling, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Goettingen, Germany
[2] Institute of Cell Biochemistry, Hannover Medical School, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

Corresponding author: Shcherbata, Halyna R. (halyna.shcherbata@mpibpc.mpg.de)

Current Opinion in Insect Science 2018, **31**:29–36

This review comes from a themed issue on **Development and regulation**

Edited by **Elisabeth Marchal** and **Maria-Dolors Piulachs**

For a complete overview see the Issue and the Editorial

Available online 19th July 2018

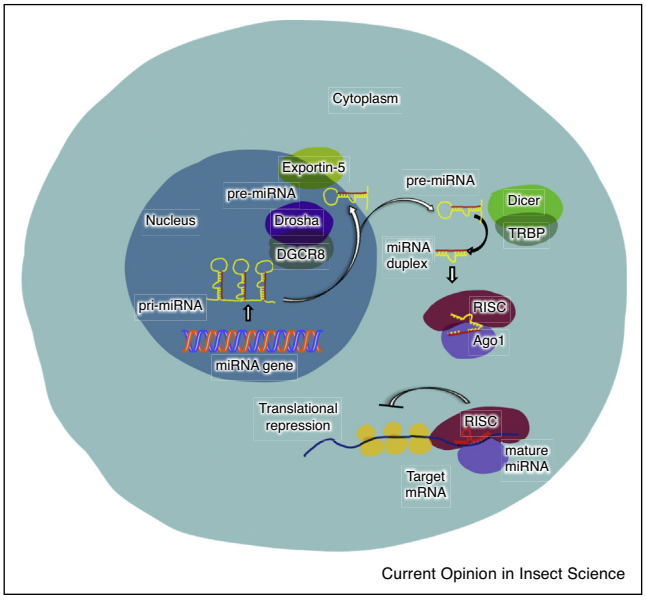
https://doi.org/10.1016/j.cois.2018.07.006

Introduction

MicroRNAs (miRNAs) are endogenous small RNAs that negatively regulate gene expression at the posttranscriptional level in a variety of eukaryotic organisms. Generation of the mature functional miRNA is a stepwise process that depends on several key RNA-binding enzymes [1–4] (Figure 1). Importantly, the efficiency of miRNA production is highly stress-dependent [5]. Since miRNA discovery less than three decades ago, it has been demonstrated that miRNAs play a role in essentially all biological processes ranging from the regulation of stemness, cell division, and differentiation to stress-dependent adjustment of cellular metabolism and organismal homeostasis [5–9,10••]. The *Drosophila* germline was used to show for the first time that the miRNA pathway is involved in the regulation of stem cell division [11]. Later it was also found that the miRNA pathway controls stem cell maintenance [12–14] and indicates stage-specific conditions for stem cell self-renewal and differentiation [15], revealing that miRNAs are crucial components of the temporally and spatially coordinated gene regulation network. These pioneering studies were based on the analysis of phenotypes caused by defects in critical enzymes required for miRNA biogenesis, such as Dicer and Drosha, absence of which would have a global effect on miRNA production (Figure 1). However, hundreds of different miRNA genes have been identified in all metazoans, of which many are phylogenetically conserved [16]. Like with any other classes of regulatory genes, in order to have a global perspective about biological roles of miRNAs, first it is important to study individual cases. Unfortunately, currently only a few miRNA–target interactions have been experimentally confirmed, and there are several reasons why studies aiming to dissect the function of a single miRNA and validate its relevant target appear to be complex.

Firstly, it remains a key difficulty to unravel specific *in vivo* functions for individual miRNAs since in well-controlled lab conditions, the majority of studied miRNA mutants are viable, fertile, and seemingly normal [17]. Secondly, while there are various databases and algorithms using physical and chemical characteristics or conserved sequences to predict miRNA–mRNA interactions, they still give large numbers of false positives [18,19]. Thirdly, bioinformatics predicts that one miRNA can target up to 200 transcripts and one mRNA can be targeted by multiple miRNAs, giving an enormous amount of combinatorial possibilities. Therefore, associating causal targets to miRNA phenotypes continues to be extremely challenging. Fourthly, the specific miRNA function is highly dependent on the presence and levels of its multiple targets which can compete for miRNA binding [20]. Therefore, the possibility of miRNA-based targeting depends not only on the expression levels of the miRNA and its target, but also on the presence of other endogenous RNAs. Finally, while the expression profile of miRNA is very specific for tissue types and developmental stages, it is also extremely sensitive to variations in organismal physiology and stress [21,22,23•,24]. Therefore, the efficiency of miRNA-based regulation even in the same cell type appears to be highly stage-dependent and condition-dependent.

Figure 1



The miRNA pathway. MiRNAs are expressed in the nucleus as many other genes by RNA polymerase II to form the primary miRNA transcripts (pri-miRNAs). The hairpin structure of the pri-miRNA is recognized by the nuclear RNase III enzyme, Drosha, which together with its partner Pasha/DGCR8, cleaves pri-miRNAs into ~70 nucleotide hairpin precursor miRNAs (pre-miRNAs). The pre-miRNA is recognized and exported to the cytoplasm by Exportin-5. In the cytoplasm, the pre-miRNA is further cleaved by another RNase III enzyme, Dicer, into a ~22 nucleotide RNA duplex, one strand of which is preferentially loaded into one of the Argonaute proteins (Ago1 in *Drosophila*). Together with Ago1 and associated proteins, the mature miRNA forms the RNA-induced silencing complexes (RISC) and guides it to the target mRNA leading to its translational repression.

Despite all these challenges, the great efforts of multiple research groups have provided a vast amount of data allowing us to gain a greater understanding of the mechanisms of miRNA-based regulation and demonstrated the importance of miRNAs in control of virtually all developmental processes. There is evidence that miRNAs act as managers of cellular homeostasis as they respond to fluctuations in environmental conditions and stresses to readjust factors ensuring cellular homeostasis. In addition, miRNA-based regulatory networks are managed via feedback-feedforward signaling. This allows the reduction of transcriptional noise and global fine-tuning of the gene expression profile to guarantee the cell fate robustness [25–27]. This is particularly important for stem cell biology since one of the key characteristics of stem cells is their capability to maintain the continuous balance between self-renewal and differentiation. Therefore, understanding the biological roles of miRNAs, particularly in stem cells, has a great potential for regenerative medicine.

Drosophila germline stem cell niche community as a model for adult stem cells

Using asymmetric cell division, stem cells acquired a remarkable potential to reproduce themselves and generate differentiating daughter cells that must convert from multi-potency to unipotency, while ceasing their self-renewal capacity. These decisions must be robustly controlled since any imbalance could lead to developmental abnormalities, defective tissue homeostasis, or cancer. In order to persist, adult stem cells must reside in a specialized location, the stem cell niche, which itself is as essential for stem cell well-being as the intrinsic stem cell functions. The niche incorporates all cellular and non-cellular constituents necessary for the adult stem cell maintenance; moreover, the stem cell niche milieu is capable to reprogram and to convert differentiated cells into stem cells [23•]. Therefore, the best way to understand stem cells is to study them in their 'home' environment. Currently, only a few stem cell niche models have emerged, among which the *Drosophila* germline stem cell niche community undoubtedly is the best studied and understood. Therefore, this review is focused on recent findings that explain the role of individual miRNAs in ovarian germline stem cells and their niches.

Thanks to systematic genetic studies in *Drosophila*, extensive transcription factor networks that coordinate germline stem cell maintenance and stem cell niche formation are well described [28,29]. In addition, it is known that in the germline, the shift between stem cell self-renewal and differentiation is controlled via interlocked feedback loops, which predominantly depend on reciprocal translational repression safeguarding this fundamental cell fate decision [30]. Moreover, it has been shown that gonads (testes and ovaries) are unusually rich in miRNAs, many of which are unique, sexually biased, and dynamically expressed [22], suggesting that miRNAs help to maintain regulatory circuits that balance the efficiencies of stem cell self-renewal and differentiation, especially in response to organismal need and environmental conditions.

The *Drosophila* ovary is a paired organ consisting of individual ovarioles, which are strings of gradually developed egg chambers. At the anterior of each ovariole resides a specialized structure called the germarium, at the apex of which the germline stem cells are located. Importantly, the germarium contains two types of ovarian stem cell niches: (i) the germline stem cell niche that maintains stem cells during the entire life of the organism and (ii) the differentiation niche that regulates the productiveness of germline differentiation. In the ovary, cells of very different origins, the germline and the soma, coexist and actively communicate. Several screens have been performed, which identified miRNAs that are expressed in the ovarian germline, soma, or both

[22,31]. However, currently, only several examples of miRNAs which cell-autonomously or cell non-autonomously affect stem cell self-renewal and differentiation have been studied.

miRNAs cell-autonomously regulate GSC maintenance and division

One example of such a miRNA is *bantam*, without which germline stem cells are as rapidly lost as in the absence of *Dicer-1* [15,31,32]. *bantam* is an unusual miRNA because, unlike most miRNA mutants, *bantam* loss-of-function mutants are lethal, suggesting that this miRNA regulates essential genes. In the imaginal discs, *bantam* supports cell proliferation and confines apoptosis [33] and in a Myc-dependent manner enhances tissue growth [34]. Moreover, the entire animal body size depends on regulation of *bantam* since it coordinates the crosstalk between steroid and insulin signaling [34]. In particular, regulation of steroid production by insulin signaling relies on the repression of *bantam* activity. Importantly, *bantam* miRNA has similar functions in the germline stem cells; it inhibits FOXO-mediated transcription of the pro-apoptotic Smac/DIA-BLO ortholog, Hid (Figure 2a). Interestingly, this regulation is used by germline stem cells (but not their daughters) to become resistant to radiation/chemical-induced apoptosis [32]. These data support previous findings which demonstrated that the miRNA pathway is required for germline stem cell maintenance [12,15] and show that there is at least one miRNA expressed in the germline stem cells to intrinsically regulate their fitness and resilience.

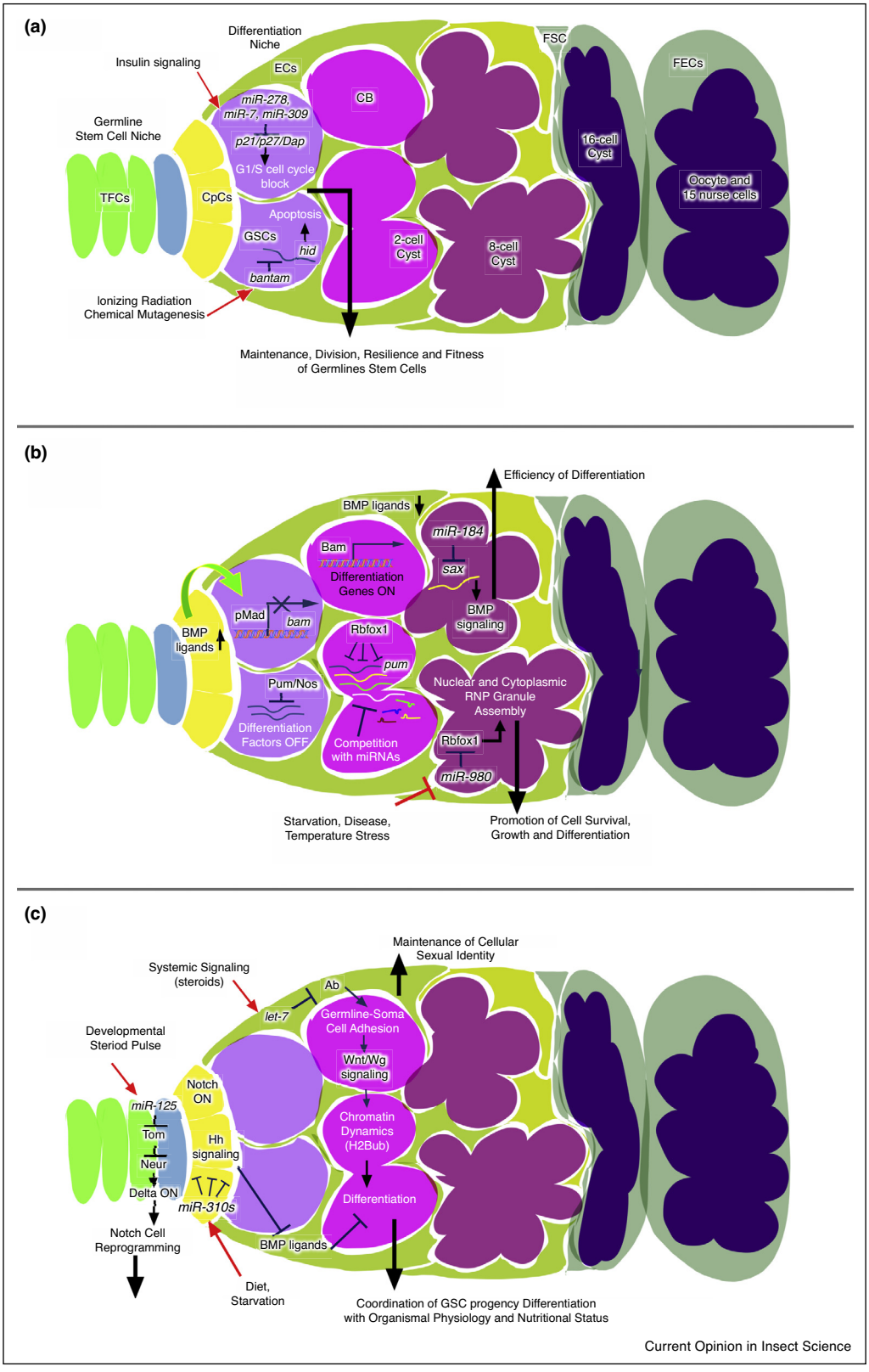
In *Drosophila*, the kinetics of adult germline stem cell division is slow and can be influenced by the environment. The miRNA pathway deficiency makes stem cells unresponsive to environmental signals that usually halt the cell cycle at the G1/S transition [11]. Germline stem cells lacking *Dicer-1* are delayed in the G1/S transition associated with the increased levels of CDK-inhibitor p21/27/Dacapo [11]. Actually, several miRNAs (*miR-278*, *miR-7* and *miR-309*, Figure 2a) that repress CDK-inhibitor p21/p27/Dacapo have been identified [35]. Apparently, the mechanisms regulating continuous self-renewal and cell cycle progression may be stem cell exclusive [36]; therefore, it is tempting to speculate that the stem cell division mode is differentially fine-tuned by miRNA-based targeting on cell cycle gate-keepers. It would be important to identify other miRNAs that influence the speed of stem cell division via adjustment of mechanisms that control stem cell sensitivity to environmental signals. Since miRNAs are also involved in tumorigenesis [37], an interesting hypothesis is that in cancer cells that also have atypical cell cycle regulation, miRNAs could play similar roles.

miRNAs cell-autonomously regulate GSC progeny differentiation

By their most general definition, stem cells are cells capable to self-renew and produce differentiating cells. Not unexpectedly, apart from being able to control stem cell self-renewal processes (maintenance and cell division), miRNAs have also been shown to be involved in control of stem cell progeny differentiation. Currently, two miRNAs that target factors required for proper ovarian germline differentiation have been identified. *miR-184* has been shown to control germline stem cell differentiation by tuning Saxophone (Sax) [38]. Sax is one of the receptors of the bone morphogenetic protein (BMP)-like receptor signaling pathway or Decapentaplegic (Dpp) signaling in *Drosophila*. The Dpp pathway chiefly governs stem cell maintenance and differentiation in the female germline [39,40]. Dpp ligands are sent from the somatic stem cell niche cells to induce Dpp signaling in the adjacent germline stem cells, which results in phosphorylation of the transcription factor Mad (Mothers against Decapentaplegic). pMad translocates to the nucleus and represses Bam, expression of which is essential for the germline differentiation (Figure 2b). In the absence of *miR-184*, Sax levels are increased, which mimics overactivation of Dpp signaling and leads to Bam repression in the germline stem cell progeny, delaying their differentiation [38].

The second described example of cell-autonomous regulation by a miRNA of an essential germline stem cell differentiation factor is the case of stress-dependent targeting of Rbfox1 by *miR-980* [41••] (Figure 2b). Rbfox1 is a multifunctional protein; in the nucleus it acts as an alternative splicing factor, while in the cytoplasm, it binds *3′UTRs* of several germline mRNAs and competes with miRNAs for their binding sites [42•,43]. In particular, Rbfox1 directly interacts with the *3′UTR* of *pumilio (pum)* mRNA and blocks its translation [42•]. Pum is an RNA-binding protein that controls germline stem cell self-renewal by translational repression of multiple germline-specific mRNAs, and its deficiency causes germline stem cell loss by differentiation [30] (Figure 2b). Thus, one of the functions of Rbfox1 is to regulate ovarian germline stem cell progeny differentiation via repression of *pum* mRNA. In addition to its RNA-binding domain, Rbfox1 contains multiple low complexity sequence domains (LCDs). Depending on its concentration, Rbfox1 promiscuously associates and induces the assembly of different types of RNA granules, such as stress granules and P-bodies in the cytoplasm or Cajal bodies and nucleoli in the nucleus [41••]. These RNA granules are produced as a result of liquid-to-liquid phase separation of LCD-containing proteins; therefore, they are also called 'liquid organelles' [44–47]. Currently, the posttranscriptional regulation of RNA metabolism in these organelles is considered to be a major stress response mechanism [5,48,49]. Upon stress, *miR-980* is downregulated

Figure 2



Examples of cell-autonomous and non-autonomous regulation of germline stem cell behavior by miRNAs.(a) Schematic of an adult germarium which contains two types of somatic GSC niches: the stem cell niche *per se,* which includes Terminal Filament Cells (TFCs, green,

and is not available for *Rbfox1* mRNA targeting, increasing Rbfox1 protein levels. This induces the widespread formation of various Rbfox1-positive liquid organelles, which modulate nuclear and cytoplasmic RNA biogenesis. This subsequently promotes cell survival upon stress [41••]. Remarkably, Rbfox1 *per se* is regulated by a miRNA and at the same time, it influences the ability of other miRNAs to interact with their targets. In addition, Rbfox1 can facilitate the assembly of various subcellular sites where mRNA processing and turnover, including mRNA–miRNA interaction, is occurring.

Recently, a novel, intriguing hypothesis of gene expression regulation called 'the Rosetta stone of a hidden RNA language' was proposed in which competing endogenous RNAs (ceRNAs), such as messenger RNAs, transcribed pseudogenes, and long noncoding RNAs use miRNA-binding sites as letters to 'talk' to each other [50]. Depending on their expression levels, they compete for miRNAs, which creates a complex network that considers the pool of various cellular RNAs as an active community that harmonizes gene expression. This communication appears to be particularly important in the control of cell fate acquisition by stem cell progeny and in cancer development [51–53]. The example of Rbfox1 and *–miR-980* interaction adds an additional layer of complexity to the ceRNA model, where Rbfox1 acts as an interferent in the hidden RNA language. Rbfox1 is targeted by miRNAs, while simultaneously competing with miRNAs for binding sites in the *3′UTRs* of different mRNAs (Figure 2b). Moreover, via Rbfox1-dependent formation of various RNP granules, it manages large scale RNA metabolism. Interestingly, the stress-dependent *miR-980* is positioned at the top of this intricate signaling cascade, which regulates gene expression under stress in the germline cells.

miRNAs cell non-autonomously regulate GSC progeny differentiation under stress

To preserve the lifelong continuity of self-renewing divisions in an environment where most of the other cells are quiescent, adult stem cells must reside in the stem cell niche. Communication between stem cells and their niches adjusts stem cell division and differentiation to organismal needs; therefore, it is logical that systemic signaling that reflects the general physiological status of the organism should be involved in coordination of stem cell self-renewal. Interestingly, it has been shown that steroid hormones act via dependent miRNAs to regulate the cellular identity of somatic cells that form the germline differentiation niche and coordinate the speed of germline differentiation [54]. This is attuned by the steroid-induced *let-7* miRNA, which via the feedback loop downregulates the transcription factor and ecdysone signaling repressor Abrupt [54,55] (Figure 2c). Depending on the strength of steroid hormone signaling that is dynamically readjusted in accordance with internal and external cues, cellular sexual identity, cell cycle mode, shape, and most importantly, adhesiveness of somatic

(Figure 2 Legend Continued) blue) and Cap Cells (CpCs, yellow) and the differentiation niche made of Escort Cells (ECs, olive). Importantly, the stem cell niche is the lifetime residence for the Germline Stem Cells (GSCs, lavender). In addition, the germarium contains somatic Follicle Stem Cells (FSC, sage) that give rise to the follicular epithelium cells (FECs, sage), which enwrap differentiating egg chambers as they pinch off the germarium. When GSCs are detached from the niche, they lose their self-renewing capacities and differentiate. Intrinsically-expressed miRNAs, *miR-278*, *miR-7* and *mir-309* regulate cyclin-dependent kinase inhibitor p21/p27/Dap. Dap binds to Cyclin E-CDK complex and renders it inactive. This promotes G1/S cell cycle block in response to dietary alterations mediated by insulin signaling, thus regulating GSC division upon dietary restriction. In order to protect the GSC pool from ionizing radiation and chemical mutagenesis, *bantam* is expressed in the GSCs to target *hid* mRNA. Hid is a conserved pro-apoptotic factor, downregulation of which prevents ionizing radiation-induced cell death. **(b)** BMP ligands are produced by the niche cells to induce BMP signaling in the GSCs, upon which the transcription factor Mad is phosphorylated. pMad represses the transcription of a differentiation factor Bam, which prevents GSC differentiation and promotes GSC maintenance. In addition, GSC maintenance is controlled at the translational level; the Pum/Nos complex binds to germline specific differentiation-promoting mRNAs, which leads to their translational repression and helps to maintain GSCs. In the differentiation niche, the levels of BMP ligands are reduced; therefore, Bam can be expressed, which allows the GSC progeny to enter the differentiation program. An intrinsically-expressed miRNA, *miR-184,* targets one of the BMP receptors, Sax, which reduces BMP signaling in the GSC daughters and permits differentiation. In the differentiating germline cells, cytoplasmic Rbfox1 binds to *3′UTRs* of several germline-specific miRNAs, including *pum* mRNA. Translational repression of *pum* decreases Pum levels, enhancing the differentiation efficiency. In addition, via binding to *3′UTRs,* Rbfox1 competes with miRNAs for mRNA targeting. Upon stress, the levels of *miR-980* that targets *Rbfox1* are reduced, leading to the increase in Rbfox1 levels followed by the widespread formation of various RNP granules, which globally controls RNA metabolism and promotes cell survival. **(c)** In response to alterations in organismal physiology, steroids induce *let-7* expression in the ECs, where it targets a negative regulator of steroid signaling, a transcription factor Abrupt (Ab). This double negative feedback loop enhances steroid signaling in the differentiation niche cells. *let-7* is required for the maintenance of the squamous epithelial cell fate and sexual identity of ECs. Due to the epithelial cell fate change, the adhesive characteristics of ECs are altered, affecting the cell adhesion between the soma and germline, which is mediated via Cadherin and Armadillo (Arm, *Drosophila* β-catenin). Arm is a Cadherin binding partner and a Wingless (Wnt/Wg) transcription factor. Since pools of Arm available for cell adhesion and Wnt/Wg signaling are interchangeable, alterations in the cell adhesion strength affect the efficiency of Wnt/Wg signaling in the germline. Wnt/Wg signaling is required to resolve the bistability of germline differentiation genes via monoubiquitination of histone 2B (H2Bub) which permits GSC progeny differentiation. Upon dietary stress, the *miR-310s* cluster of miRNAs is expressed in the somatic niches. *miR-310s* target multiple components of Hedgehog (Hh) signaling, which provides a quick and robust dietary response. Activation of Hh signaling is required to restrict production of BMP ligands in the differentiation niche, licensing GSC progeny differentiation. During development, the steroid pulse-induced *miR-125* is expressed in the posterior TFC. It targets Twin of m4 (Tom) which negatively regulates the ability of Neuralized (Neur) to activate the Notch ligand, Delta. Activation of Delta, achieved in response to *miR-125* induction, reprograms the status of this TFC from Notch signal-receiving to Notch signal-sending. Activated Delta sent from this cell induces the Notch signal-receiving cell fate in the adjacent CpCs precursors via hexagonal tiling. This results in the establishment of the stereotypical GSC niche.

cells in the differentiation niche are modified [22,54,56]. Since somatic and germline cells in the ovary are attached via the cadherin-dependent homophilic cell adhesion, cadherin levels must correlate on the membranes of the adjacent cells. Therefore, alterations in somatic cell adhesiveness have a direct effect on cell adhesion proteins expressed in the germline. Cadherins bind signaling molecules, for example Armadillo (*Drosophila* β-catenin), which concurrently with its role in the cell adhesion, acts a transcriptional factor of Wingless (Wg or Wnt in vertebrates) signaling. Therefore, steroid signaling from the soma via direct cell contacts adjusts Wg signaling in the germline, the efficacy of which has a positive effect on germline differentiation. In particular, Wg-based regulation occurs via chromatin modifications, such as histone H2B monoubiquitination, which permits the germline stem cell progeny to begin the differentiation program [54]. These data demonstrate a model in which, in response to external cues, the soma influences the tempo of germline differentiation via miRNAs.

Another interesting aspect is that in gonads, the steroid-induced miRNA *let-7* maintains the sexual identity of the somatic cells [22]. Although the signaling cascade that determines sexual identity has been comprehensively studied and is well characterized, the idea that specific signals are required to preserve sexual identity during adult life is quite novel. The data from *Drosophila* establish miRNAs as important managers shaping sexual dimorphism and form the foundation of future work addressing the functions of miRNAs in maintenance of cellular sexual identity.

Also, miRNAs have been shown to adjust cell signaling in order to shape adult oogenesis to fluctuating diet. In particular, the *miR-310s* cluster targets multiple factors of the diet-dependent Hh pathway to guarantee its quick and robust downregulation upon starvation [10••] (Figure 2c). Hh signaling has multiple means to control ovarian germline stem cell division and differentiation in a cell non-autonomous manner: (i) Hh ligand is produced by the stem cell niche cells, which deliver it to the differentiation niche cells to manage the GSC population; (ii) activated Hh signaling restricts production of BMP ligands in both niches to permit germline stem cell progeny differentiation; (iii) the amount of Hh ligand is sensed by follicle stem cells (FSCs) to coordinate the speed of follicular epithelium cell division with germline differentiation [10••,57,58•]. In the somatic stem cell niche cells, the *miR-310s* expression is highly dynamic and nutrition-sensitive, and it is currently undetermined how *miR-310s* expression is adjusted in response to nutritional deficit. However, in other tissues, their expression is modulated by stress-sensitive nitric oxide signaling that nitrosylates histone deacetylases, which positively influences gene expression via histone modifications [21,59,60]. Hh signaling is a principal pathway known to regulate dietary stress-response in stem cells [61,62] and interestingly, the *miR-310s* act upstream of this signaling. These data demonstrate that miRNAs transduce the information about the nutritional status of an organism to an essential signaling pathway, which via stem cell niche signaling controls stem cell behavior. This implies that miRNAs are promising agents capable to control stem cells upon fluctuating dietary conditions.

miRNAs cell-autonomously regulate GSC niche formation

Not only do miRNAs play important roles in the adjustment of germline stem cell maintenance and differentiation during adulthood, a miRNA has been identified that influences the process of stem cell niche formation. Induced by the steroid pulse, *miR-125* acts as an intermediary between temporal steroid and spatial Notch signaling to aid in the process of the stereotypical germline stem cell niche assembly (Figure 2c). Interestingly, the steroid–*miR-125*–Notch signaling cascade is used to reprogram Notch signaling status of the posterior terminal filament cell, which becomes Delta-sending and activates Notch signaling in the adjacent CpC precursors [63••]. MiRNA-mediated Notch signaling reprogramming allows TFC to become the inducer of the hexagonal stem cell niche pattern. Since hexagonal tiling is the most stable pattern in nature, this mechanism optimizes the stem cell niche architecture [63••]. This is extremely important, since niche cells are not renewable and at the same time, they must assemble into a structure that could function as a lifelong residence for stem cells, and notably, a miRNA is instrumental in this critical process of stem cell niche establishment.

Conclusions

In summary, the research about miRNA functions in *Drosophila* stem cells and their niches provides new knowledge regarding the mechanisms and logic of miRNA-based regulation, which has great potential for regenerative medicine applications. By studying miRNAs in the germline, we have not only identified additional players in signaling cascades regulating stem cell behavior but have also discovered new mechanisms utilized by stem cells and their niches that allow them to be maintained as a properly functioning community that is easily adjustable to organismal needs.

Funding

This work was supported by the Max Planck Society, EMBO YIP, VolkswagenStiftung and Behrens-Weise Foundation.

Conflict of interest statement

Nothing declared.

Acknowledgements

I would like to thank Travis Carney, Andriy Yatsenko and the anonymous reviewers for helpful suggestions and critical reading of the manuscript.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as

• of special interest
•• of outstanding interest

1. Connerty P, Ahadi A, Hutvagner G: **RNA binding proteins in the miRNA pathway**. *Int J Mol Sci* 2015, **17**.

2. Creugny A, Fender A, Pfeffer S: **Regulation of primary microRNA processing**. *FEBS Lett* 2018.

3. Wu K, He J, Pu W, Peng Y: **The role of exportin-5 in microRNA biogenesis and cancer**. *Genomics Proteom Bioinform* 2018, **16**:120-126.

4. Czech B, Hannon GJ: **Small RNA sorting: matchmaking for Argonautes**. *Nat Rev Genet* 2011, **12**:19-31.

5. Kucherenko MM, Shcherbata HR: **miRNA targeting and alternative splicing in the stress response — events hosted by membrane-less compartments**. *J Cell Sci* 2018, **131**.

6. Barrio L, Dekanty A, Milan M: **MicroRNA-mediated regulation of Dp53 in the Drosophila fat body contributes to metabolic adaptation to nutrient deprivation**. *Cell Rep* 2014, **8**:528-541.

7. Leung AK, Sharp PA: **MicroRNA functions in stress responses**. *Mol Cell* 2010, **40**:205-215.

8. Ross SA, Davis CD: **MicroRNA, nutrition, and cancer prevention**. *Adv Nutr* 2011, **2(6)**:472-485.

9. Carthew RW, Agbu P, Giri R: **MicroRNA function in *Drosophila melanogaster***. *Semin Cell Dev Biol* 2017, **65**:29-37.

10. •• Cicek IO, Karaca S, Brankatschk M, Eaton S, Urlaub H, Shcherbata HR: **Hedgehog signaling strength is orchestrated by the mir-310 cluster of microRNAs in response to diet**. *Genetics* 2016, **202**:1167-1183.

In this paper authors proposed a new roadmap for miRNA function analysis, which they used to find that in*Drosophila*, the *miR-310s* regulate metabolism. *mir-310s* mutants store fats and display various diet-dependent physiological defects in addition to the abnormal gene expression. The *mir-310s* concurrently target several regulatory factors of the Hedgehog signaling pathway (Rab23, DHR96, and Ttk) to sharpen dietary response. These data are the first to report that miRNAs transduce nutritional signals to an essential signaling pathway.

11. Hatfield SD, Shcherbata HR, Fischer KA, Nakahara K, Carthew RW, Ruohola-Baker H: **Stem cell division is regulated by the microRNA pathway**. *Nature* 2005, **435**:974-978.

12. Jin Z, Xie T: **Dcr-1 maintains Drosophila ovarian stem cells**. *Curr Biol* 2007, **17**:539-544.

13. Park JK, Liu X, Strauss TJ, McKearin DM, Liu Q: **The miRNA pathway intrinsically controls self-renewal of Drosophila germline stem cells**. *Curr Biol* 2007, **17**:533-538.

14. Yang L, Chen D, Duan R, Xia L, Wang J, Qurashi A, Jin P, Chen D: **Argonaute 1 regulates the fate of germline stem cells in Drosophila**. *Development* 2007, **134**:4265-4272.

15. Shcherbata HR, Ward EJ, Fischer KA, Yu JY, Reynolds SH, Chen CH, Xu P, Hay BA, Ruohola-Baker H: **Stage-specific differences in the requirements for germline stem cell maintenance in the Drosophila ovary**. *Cell Stem Cell* 2007, **1**:698-709.

16. Bartel DP, Chen CZ: **Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs**. *Nat Rev Genet* 2004, **5**:396-400.

17. Lai EC: **Two decades of miRNA biology: lessons and challenges**. *RNA* 2015, **21**:675-677.

18. Shukla V, Varghese VK, Kabekkodu SP, Mallya S, Satyamoorthy K: **A compilation of Web-based research tools for miRNA analysis**. *Brief Funct Genomics* 2017, **16**:249-273.

19. Roberts JT, Borchert GM: **Computational prediction of microRNA target genes, target prediction databases, and web resources**. *Methods Mol Biol* 2017, **1617**:109-122.

20. Ulitsky I: **Interactions between short and long noncoding RNAs**. *FEBS Lett* 2018 http://dx.doi.org/10.1002/1873-3468.13085.

21. Marrone AK, Edeleva EV, Kucherenko MM, Hsiao NH, Shcherbata HR: **Dg-Dys-Syn1 signaling in Drosophila regulates the microRNA profile**. *BMC Cell Biol* 2012, **13**:26.

22. Fagegaltier D, Konig A, Gordon A, Lai EC, Gingeras TR, Hannon GJ, Shcherbata HR: **A genome-wide survey of sexually dimorphic expression of Drosophila miRNAs identifies the steroid hormone-induced miRNA let-7 as a regulator of sexual identity**. *Genetics* 2014, **198**:647-668.

23. • Kasper DM, Moro A, Ristori E, Narayanan A, Hill-Teran G, Fleming E, Moreno-Mateos M, Vejnar CE, Zhang J, Lee D *et al.*: **MicroRNAs establish uniform traits during the architecture of vertebrate embryos**. *Dev Cell* 2017, **40**:552-565 e555.

Authors performed a single-cell resolution screen of zebrafish embryonic blood vessels upon mutagenesis of various miRNA. They found that even though embryos deficient for certain miRNAs develop a vascular trait similar to wild-type, a dramatic increase in phenotypic heterogeneity is present. Such abnormal trait variance in miRNA mutants demonstrates that the absence of miRNAs sensitizes vascular system development to environmental stress. These data show for the first time that miRNAs protect tissue development against phenotypic variability.

24. Edeleva EV, Shcherbata HR: **Stress-induced ECM alteration modulates cellular microRNAs that feedback to readjust the extracellular environment and cell behavior**. *Front Genet* 2013, **4**:305.

25. Yatsenko AS, Shcherbata HR: **Drosophila miR-9a targets the ECM receptor Dystroglycan to canalize myotendinous junction formation**. *Dev Cell* 2014, **28**:335-348.

26. Posadas DM, Carthew RW: **MicroRNAs and their roles in developmental canalization**. *Curr Opin Genet Dev* 2014, **27**:1-6.

27. Liufu Z, Zhao Y, Guo L, Miao G, Xiao J, Lyu Y, Chen Y, Shi S, Tang T, Wu CI: **Redundant and incoherent regulations of multiple phenotypes suggest microRNAs' role in stability control**. *Genome Res* 2017, **27**:1665-1673.

28. Greenspan LJ, de Cuevas M, Matunis E: **Genetics of gonadal stem cell renewal**. *Annu Rev Cell Dev Biol* 2015, **31**:291-315.

29. Flora P, McCarthy A, Upadhyay M, Rangan P: **Role of chromatin modifications in Drosophila germline stem cell differentiation**. *Results Probl Cell Differ* 2017, **59**:1-30.

30. Slaidina M, Lehmann R: **Translational control in germline stem cell development**. *J Cell Biol* 2014, **207**:13-21.

31. Neumuller RA, Betschinger J, Fischer A, Bushati N, Poernbacher I, Mechtler K, Cohen SM, Knoblich JA: **Mei-P26 regulates microRNAs and cell growth in the Drosophila ovarian stem cell lineage**. *Nature* 2008, **454**:241-245.

32. Xing Y, Su TT, Ruohola-Baker H: **Tie-mediated signal from apoptotic cells protects stem cells in *Drosophila melanogaster***. *Nat Commun* 2015, **6**:7058.

33. Brennecke J, Hipfner DR, Stark A, Russell RB, Cohen SM: **Bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila**. *Cell* 2003, **113**:25-36.

34. Herranz H, Hong X, Perez L, Ferreira A, Olivieri D, Cohen SM, Milan M: **The miRNA machinery targets Mei-P26 and regulates Myc protein levels in the Drosophila wing**. *EMBO J* 2010, **29**:1688-1698.

35. Yu J.Y., Reynolds SH, Hatfield SD, Shcherbata HR, Fischer KA, Ward EJ, Long D, Ding Y, Ruohola-Baker H: **Dicer-1-dependent Dacapo suppression acts downstream of insulin receptor in regulating cell division of Drosophila germline stem cells**. *Development* 2009, **136**:1497-1507.

36. Shcherbata HR, Hatfield S, Ward EJ, Reynolds S, Fischer KA, Ruohola-Baker H: **The microRNA pathway plays a regulatory role in stem cell division**. *Cell Cycle* 2006, **5**:172-175.

37. Vannini I, Fanini F, Fabbri M: **Emerging roles of microRNAs in cancer**. *Curr Opin Genet Dev* 2018, **48**:128-133.

38. Iovino N, Pane A, Gaul U: **miR-184 has multiple roles in Drosophila female germline development**. *Dev Cell* 2009, **17**:123-133.

39. Song X, Wong MD, Kawase E, Xi R, Ding BC, McCarthy JJ, Xie T: **Bmp signals from niche cells directly repress transcription of a differentiation-promoting gene, bag of marbles, in germline stem cells in the Drosophila ovary**. *Development* 2004, **131**:1353-1364.

40. Chen D, McKearin DM: **A discrete transcriptional silencer in the bam gene determines asymmetric division of the Drosophila germline stem cell**. *Development* 2003, **130**:1159-1170.

41. •• Kucherenko MM, Shcherbata HR: **Stress-dependent miR-980 regulation of Rbfox1/A2bp1 promotes ribonucleoprotein granule formation and cell survival**. *Nat Commun* 2018, **9**:312.
Authors found that Rbfox1 is an LCD-containing protein which is capable to form liquid droplets and amyloid-like fibers and indiscriminately assemble various nuclear and cytoplasmic RNP granules. Rbfox1 is regulated by miR-980, which is a stress-dependent miRNA that buffers Rbfox1 levels via binding to its alternative extended 3'UTRs. Upon stress, miR-980 is downregulated, which leads to increased Rbfox1 levels and extensive formation of different RNP granules, promoting cell survival. Authors found that human RBFOX proteins also have multiple LCDs and can join various membrane less organelles, which may explain a wide variety of RBFOX-linked diseases in humans.

42. • Carreira-Rosario A, Bhargava V, Hillebrand J, Kollipara RK, Ramaswami M, Buszczak M: **Repression of pumilio protein expression by Rbfox1 promotes germ cell differentiation**. *Dev Cell* 2016, **36**:562-571.
Authors discovered that cytoplasmic Drosophila Rbfox1 represses the translation of germline-specific mRNAs via binding to (U)GCAUG elements within 3'UTRs. In particular, Rbfox1 translationally silences pumilio mRNA. Rbfox1 loss or Pum overexpression blocks germline differentiation. These results show a new splice-independent role for cytoplasmic Rbfoxin regulation of the translation of specific target mRNAs. This function ensures that the germline stem cell progeny continue to differentiate and do not revert back to the stem cell-like status.

43. Lee JA, Damianov A, Lin CH, Fontes M, Parikshak NN, Anderson ES, Geschwind DH, Black DL, Martin KC: **Cytoplasmic Rbfox1 regulates the expression of synaptic and autism-related genes**. *Neuron* 2016, **89**:113-128.

44. Feric M, Vaidya N, Harmon TS, Mitrea DM, Zhu L, Richardson TM, Kriwacki RW, Pappu RV, Brangwynne CP: **Coexisting liquid phases underlie nucleolar subcompartments**. *Cell* 2016, **165**:1686-1697.

45. Hyman AA, Weber CA, Julicher F: **Liquid–liquid phase separation in biology**. *Annu Rev Cell Dev Biol* 2014, **30**:39-58.

46. Lin Y, Protter DS, Rosen MK, Parker R: **Formation and maturation of phase-separated liquid droplets by RNA-binding proteins**. *Mol Cell* 2015, **60**:208-219.

47. Alberti S, Carra S: **Quality control of membraneless organelles**. *J Mol Biol* 2018 http://dx.doi.org/10.1016/j.jmb.2018.05.013.

48. Mateju D, Franzmann TM, Patel A, Kopach A, Boczek EE, Maharana S, Lee HO, Carra S, Hyman AA, Alberti S: **An aberrant phase transition of stress granules triggered by misfolded protein and prevented by chaperone function**. *EMBO J* 2017, **36**:1669-1687.

49. Courchaine EM, Lu A, Neugebauer KM: **Droplet organelles?** *EMBO J* 2016, **35**:1603-1612.

50. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP: **A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?** *Cell* 2011, **146**:353-358.

51. Twayana S, Legnini I, Cesana M, Cacchiarelli D, Morlando M, Bozzoni I: **Biogenesis and function of non-coding RNAs in muscle differentiation and in Duchenne muscular dystrophy**. *Biochem Soc Trans* 2013, **41**:844-849.

52. Giza DE, Vasilescu C, Calin GA: **MicroRNAs and ceRNAs: therapeutic implications of RNA networks**. *Expert Opin Biol Ther* 2014, **14**:1285-1293.

53. Chan JJ, Tay Y: **Noncoding RNA:RNA regulatory networks in cancer**. *Int J Mol Sci* 2018, **19**.

54. Konig A, Shcherbata HR: **Soma influences GSC progeny differentiation via the cell adhesion-mediated steroid-let-7-Wingless signaling cascade that regulates chromatin dynamics**. *Biol Open* 2015, **4**:285-300.

55. Kucherenko MM, Barth J, Fiala A, Shcherbata HR: **Steroid-induced microRNA let-7 acts as a spatio-temporal code for neuronal cell fate in the developing Drosophila brain**. *EMBO J* 2012, **31**:4511-4523.

56. Konig A, Yatsenko AS, Weiss M, Shcherbata HR: **Ecdysteroids affect Drosophila ovarian stem cell niche formation and early germline differentiation**. *EMBO J* 2011, **30**:1549-1562.

57. Rojas-Rios P, Guerrero I, Gonzalez-Reyes A: **Cytoneme-mediated delivery of hedgehog regulates the expression of bone morphogenetic proteins to maintain germline stem cells in Drosophila**. *PLoS Biol* 2012, **10**:e1001298.

58. • Huang J, Reilein A, Kalderon D: **Yorkie and Hedgehog independently restrict BMP production in escort cells to permit germline differentiation in the Drosophila ovary**. *Development* 2017, **144**:2584-2594.
Authors found that both Hedgehog (Hh) and Hippo/Yki limit production of BMP ligands in ECs to non-autonomously control germline differentiation. However, the Hedgehog and Hippo/Yki signaling pathways have independent functions in limiting BMP ligand production. Loss of Yki has a more profound effect than loss of Hh signaling and induces a different BMP ligand. Having multiple mechanisms of regulation of the BMP ligand production in the differentiation niche cells is important to control the efficiency of germline differentiation.

59. Marrone A.K., Shcherbata HR: **Dystrophin orchestrates the epigenetic profile of muscle cells via miRNAs**. *Front Genet* 2011, **2**:64.

60. Yatsenko AS, Marrone AK, Shcherbata HR: **miRNA-based buffering of the cobblestone-lissencephaly-associated extracellular matrix receptor dystroglycan via its alternative 3'-UTR**. *Nat Commun* 2014, **5**:4906.

61. Hartman TR, Strochlic TI, Ji Y, Zinshteyn D, O'Reilly AM: **Diet controls Drosophila follicle stem cell proliferation via Hedgehog sequestration and release**. *J Cell Biol* 2013, **201**:741-757.

62. Horner MA, Pardee K, Liu S, King-Jones K, Lajoie G, Edwards A, Krause HM, Thummel CS: **The Drosophila DHR96 nuclear receptor binds cholesterol and regulates cholesterol homeostasis**. *Genes Dev* 2009, **23**:2711-2716.

63. •• Yatsenko AS, Shcherbata HR: **Stereotypical architecture of the stem cell niche is spatiotemporally established by miR-125-dependent coordination of Notch and steroid signaling**. *Development* 2018, **145**.
Authors show that the status of Notch signaling in the cell can be reprogrammed during Drosophila ovarian germline stem cell niche formation. This is coordinated via steroid-induced miR-125, which targets a negative regulator of Notch signaling, Tom. Thus, in order to establish the stereotypical stem cell niche which will be responsible for a lifetime maintenance of GSCs, miR-125 acts as a coordinator between spatial Notch and temporal steroid signaling.