Review

Fruit fly research in China

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ABSTRACT

Served as a model organism over a century, fruit fly has significantly pushed forward the development of global scientific research, including in China. The high similarity in genomic features between fruit fly and human enables this tiny insect to benefit the biomedical studies of human diseases. In the past decades, Chinese biologists have used fruit fly to make numerous achievements on understanding the fundamental questions in many diverse areas of biology. Here, we review some of the recent fruit fly studies in China, and mainly focus on those studies in the fields of stem cell biology, cancer therapy and regeneration medicine, neurological disorders and epigenetics.

1. Introduction

*Drosophila melanogaster* (known as fruit fly) is a fascinating model organism and has been extensively used in scientific research for over one hundred years (Bellen et al., 2010). Fruit fly has several practical features that are suitable for laboratory study, including a short life cycle, readily culture and maintenance, and a low number of chromosomes, which enable fruit fly a commonly used model organism in biological research, particularly in genetics and developmental biology. Notably, the genome sequencing of fruit fly reveals that approximately 75% of known human disease genes have orthologs in fruit fly (Adams et al., 2000; Reiter et al., 2001). Thereafter, fruit fly has been greatly benefited the biomedical studies in cancer, immunity and neurological diseases (Bellen et al., 2010; Gonzalez, 2013; Buchon et al., 2014).

After rediscovering Mendelian inheritance in 1900, Professor Thomas Hunt Morgan (1866–1945), an American evolutionary biologist and geneticist who won the Nobel Prize in Physiology or Medicine in 1933, started to use the fruit fly for genetic researches and demonstrated that genes are located on chromosomes, which laid the fundamental basis of the modern genetics. Some of Morgan’s Chinese students performed excellent research and are later known in China as the founders of their fields. Among them, Professor Ju-Chi (Ruqi) Li (1895–1991) and Professor Chia-Chen (Jiazhen) Tan (1909–2008) significantly contributed to the genetic research and acted as the pioneers and founders of modern genetics in China. Professor Ruqi Li was the first Chinese PhD graduate from Morgan’s lab and published his first fly paper showing how chromosomal mutation influences fruit fly developments in Genetics (Li, 1927). Until now, this research article is still recognized as a classic work in developmental genetics. Professor Jiazhen Tan was trained by Professor Li in China and then in Morgan’s lab, conducting many influential works using fruit flies and lady-bird (Tan, 1942; Tan, 1946). Professor Jiazhen Tan was later called “the Chinese Morgan.”

Inspired by the pioneers in the field of genetics, in the past decades, the younger generation of Chinese biologists have used fruit fly to make numerous achievements on understanding the fundamental questions in many diverse areas of biology, from developmental biology to neuroscience. In this review, we cover some of the recent fruit fly studies in China, and mainly focus on those studies in the fields of stem cell biology, cancer therapy and regeneration medicine, neurological disorders and epigenetics.

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2. Stem cell biology

2.1. Germline stem cells

Drosophila ovary provides an excellent model system for studying a variety of fascinating biological processes, such as stem cell regulation, germline-soma interaction and meiosis regulation. Drosophila oogenesis initiates at the tip of the germarium, where a germline stem cell (GSC) divides asymmetrically to produce a daughter GSC and a cystoblast (CB). CBs eventually develop into mature eggs and sustain oogenesis. Thus, the balance between self-renewal and differentiation of GSCs is the critical regulatory step for germline development. Over the last ten years, several Chinese research groups have used the Drosophila ovary as model to study the molecular mechanisms underlying GSC fate determination, and characterized a number of factors that cell-autonomously regulate self-renewal and differentiation of GSCs (Yang et al., 2007; Jiang et al., 2008; Zhao et al., 2008; Chen et al., 2009; Yang et al., 2009; Sun et al., 2010; Li et al., 2015a; Sun et al., 2015; Ji et al., 2017; Li et al., 2017b; Liu et al., 2017c; Chen et al., 2018a). These studies suggested that the miRNA pathway, ubiquitin-mediated cell-cycle program, TSC1/T2 tumor suppressor complex and others are cell-autonomously required for GSC maintenance. In addition to intrinsic factors, the maintenance and differentiation of GSCs also require factors involved in the interaction between GSCs and their surrounding stalk cells (also called stem cell niches). Zhaohui Wang’s group found gypsyic Dally functions as the co-factor of Dpp to establish short-range BMP (bone morphogenetic protein) signaling in GSC, thus maintaining GSC identity (Guo and Wang, 2009). Dahua Chen’s group showed that GSCs and CBs can differentially respond to niche BMP signal, thus forming a steep gradient of BMP signaling response that controls the GSC fate (Xia et al., 2010). Moreover, Dahua Chen and Yi Tao’s groups performed mathematic modeling analyses and revealed a bistable behavior of the feedback-loop system in controlling fate determination of GSCs (Xia et al., 2012). It is worth to note that the Bam protein has been demonstrated to play a critical role in promoting early germ cell differentiation in the Drosophila ovary; however, its biochemical nature has long been a mystery. Dahua Chen’s group recently showed that Bam functions as an ubiquitin-associated protein, and cooperates with Otu (ovarian tumor) to deubiquitinate and stabilize Cyclin A (CycA), thereby balancing GSC self-renewal and differentiation (Ji et al., 2017).

In addition to ovary, Chinese biologists have used Drosophila testis as a model to study germline development, stem cell maintenance and proliferation, and identified a number of RNA-binding proteins, noncoding RNAs and enzymes that regulate Drosophila spermatogenesis (Zhang et al., 2009; Zhao et al., 2013; Chen et al., 2014; Wen et al., 2016; Wu et al., 2016; Yu et al., 2016; Shan et al., 2017). These studies made significant contributions to the field of germline development.

2.2. Intestinal stem cells

Intestinal stem cells (ISCs) in the Drosophila midgut provide a simple and genetically tractable system to understand the basic principles that control adult stem cell. Each ISC periodically divides to generate a daughter enteroblast (EB), which then differentiates further into one of the two cell types: enterocyte and enteroendocrine cells. Rongwen Xi’s group made important progress on the mechanisms underlying cell fate decision during ISC differentiation. They showed that the visceral muscle cells surrounding the intestinal epithelium constitute a regulatory niche for ISCs by producing multiple secreted signal molecules, including Wingless (Wg) and epidermal growth factor (EGF)-like factors, which act on ISCs to promote ISC maintenance and proliferation (Lin et al., 2008, 2010; Xu et al., 2011). Moreover, they identified Ttk69 as a master repressor of enteroendocrine cell fate (Wang et al., 2015a). By studying the regulation of a cell fate inducer Scute, Rongwen Xi’s group found that transient induction of Scute in ISCs by feedback regulations directs the generation of enteroendocrine cells from ISCs, indicating that transcription factor oscillation in stem cells may act as a timer for cell fate decision of multipotent stem cells (Chen et al., 2018b).

ISCs are very resistant to apoptotic inducers, but are not able to be regenerated after all the progenitors are ablated (Zhang et al., 2015; Ma et al., 2016). To ensure tissue homeostasis, the proliferation and differentiation of ISCs are tightly regulated by extrinsic and intrinsic factors at multiple levels. Xinhua Lin’s group showed that the respiratory organ, trachea, produces Dpp (Decapentaplegic, homolog of BMP) ligands, which activate Dpp signaling in enterocytes (ECs) to restrict ISCs from excessive proliferation (Li et al., 2013b). They found that Dpp signaling in ECs or Dpp production in tracheal cells, ISCs undergo unrestricted proliferation and produce excessive amount of ISC-like cells and progeny, resembling human juvenile polyposis (JPP) syndrome (Haramis, 2004; Li et al., 2013b). These findings demonstrated the significance of inter-organ communication in tissue homeostasis maintenance.

Moreover, Xinhua Lin’s group found that Debra (Dbr), an intrinsic factor, maintains midgut homeostasis by negatively regulating Hedgehog (Hh) signaling in ISCs (Li et al., 2014b). Dbr mediates the degradation of Cubitus interruptus (Ci), the transcription activator in the Hh signaling pathway, thereby restricting Hh signaling from excessive activation in ISCs (Dai et al., 2003; Li et al., 2014b). Additionally, they found that another intrinsic factor, Windpipe (Wdp), maintains midgut homeostasis (Ren et al., 2015). Wdp interacts with the receptor Domeless and promotes its internalization for subsequent lysosomal degradation, forming a negative feedback loop to regulate the duration of Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling activation, thereby maintaining midgut homeostasis (Ren et al., 2015).

Given the similarities in ISc regulation between flies and mammals, the fly midgut system can also be used to model human diseases, such as intestinal tumorigenesis. Rongwen Xi’s group has demonstrated the parallel and cooperative roles for Wnt and EGF signaling during loss of adenomatous polyposis coli-induced intestinal tumors (Wang et al., 2013). They also identified a Sox21a-mediated feedback amplification loop between ISCs and their immediate daughter progenitor cells, which is essential for intestinal regeneration and tumorigenesis (Chen et al., 2016). These new findings may help to understand tissue regeneration and the disease mechanisms in mammals, including human.

2.3. Renal and nephric stem cells

The malpighian tubules (MTs or fly kidney) of adult Drosophila are maintained by renal and nephric stem cells (RNSCs) through self-renewing divisions; however, the underlying mechanisms of how RNSC proliferation and differentiation are regulated remain unclear. Zhourhua Li and Xinhua Lin’s groups showed that EGF/MAPK signaling is dispensable for the maintenance of RNSCs, but required for the proliferation of RNSCs in vivo (Li et al., 2015c). EGF/MAPK (mitogen-activated protein kinases) signaling functions independently of JAK/STAT signaling to regulate RNSC proliferation. Meanwhile, dMyc and CycE are found to partially mediate EGF/MAPK signaling activity in RNSCs (Li et al., 2015c). They have further revealed that Notch signaling is essential for...
RNSC self-renewal and differentiation (Li et al., 2014a). Notch receptor is expressed in both RNSCs and renalblasts (RBs), the immediate daughters of RNSC divisions, while the ligand Delta is expressed at high levels in RNSCs, which serves as a RNSC marker. RNSC-expressed Delta initiates Notch signal activation in RBs to promote cell cycle exit and differentiation, similar to its function in the midgut. Surprisingly, Delta is also required autonomously in RNSCs to maintain stem cell fate. Mechanistically, RNSC-expressed Delta inhibits Notch signal activation in RNSCs via the “cis-inhibition” process (Li et al., 2014a). These data demonstrate that differential Notch activity is required in RNSCs and RBs for their maintenance and differentiation, respectively (Li et al., 2014a).

2.4. Neural stem cell

Asymmetric division of a neural stem cell distinguishes a stem cell and its differentiating sibling, and thus plays vital roles in maintaining homeostasis and preventing carcinogenesis. So far, however, the mechanisms that consolidate and lock in such initial fate bias remain obscure. Conceivably, a signaling amplification mechanism could be employed in the stem cells or progenitors to accelerate the transition phase between the initial cell fate decision and the ultimate cell fate commitment. Yan Song’s group has recently implicated the Super Elongation Complex (SEC), which controls transcription elongation checkpoint, in driving fly neural stem cell fate commitment (Liu et al., 2017a). SEC is highly enriched in the neural stem cells, where it interacts directly and intimately with the Notch signaling pathway in a self-reinforcing feedback loop for timely stem cell fate lock-in. Their studies revealed an SEC-mediated intracellular amplifier mechanism in ensuring robustness and precision in stem cell fate commitment, which explains the highly frequent association of SEC over-activation with human cancers. More recently, Yan Song’s group also showed that the retromer complex could directly regulate Notch receptor retrograde trafficking in neuroblast lineages, and ensure the unidirectional Notch signaling from neural progenitors to neuroblasts. Their results unveil a safeguard mechanism of retromer, where it can act as a bomb squad to specifically retrieve and disarm potentially harmful Notch receptors in a timely manner to prevent abnormal Notch activation-induced neural progenitor dedifferentiation and brain tumor formation (Li et al., 2018).

3. Developmental signaling, cancer therapy and regeneration

3.1. Hippo signaling pathway

The Hippo signaling pathway controls the organ size, tissue homeostasis, repair and regeneration by inhibiting cell proliferation, promoting apoptosis and stem cell maintenance. Malfunction of the Hippo pathway results in various diseases including cancers, it is thus a potential target for cancer therapy and regeneration medicine. Lei Zhang’s group has been focusing on investigating the activation of Smoothened and its downstream potential of various Hh-related diseases (Xiong et al., 2015). Two transmembrane proteins, Patched (Ptc) receptor and Smoothened (Smo) signal transducer, are involved in an unusual reception system of Hh signaling. A large number of studies have been focusing on investigating the activation of Smoothened and its downstream signal transduction; however, little is known about the regulation of Patched receptor. Dahua Chen’s group showed that ubiquitin E3 ligase Smurf directly targets Patched for ubiquitination to regulate Hh signaling. Mathemetic modeling analysis suggests that a bidirectional control involving Smurf and Patched is important for signal-receiving cells to precisely interpret external signals, thereby maintaining Hh signaling reliability (Huang et al., 2013b).

The Cubitus interruptus (Ci)/Gli transcription factors can be degraded either completely or partially from a full-length form (Ci155/Gli1) to a truncated repressor (Ci75/Gli2). Yun Zhao’s group has identified Ter94 (Drosophila valosin-containing protein) ATPase as a novel stability regulator of Ci by targeting K11-linked ubiquitinated Ci for the selective partial degradation (Zhang et al., 2013e). They also identified ubiquitin specific peptide 7 (Usp7) as a novel deubiquitinase of Ci to remove ubiquitin and stabilize Ci in the presence of Hh (Zhou et al., 2015). They confirmed that Ter94-mediated Ci selective degradation and Usp7-Ci regulation axis is conserved in mammals. Moreover, they identified Atrophin as a novel repressor of the Hh signaling pathway by interacting with the N terminus of Ci repressor (CiP) and enhancing the repression effects in a CiP dependent manner. They demonstrated that Atrophin can interact with a histone deacetylase, Rpd3 (Drosophila homolog of histone deacetylase 1) and recruit it to the CiP binding sites resulting in local histone H3 deacetylation, which experimentally clarifies how Ci regulates its downstream target genes (Zhang et al., 2013d). In addition, Yun Zhao’s group has explored the biological functions of Hh signaling pathway: they demonstrated that sumoylation of Ci is required for Hh signaling-mediated cyst stem cell proliferation in the testis (Lv et al., 2016), and neuronal Hh ligands are important for ISC differentiating toward ECs (Han et al., 2015).

The heart is one of the first functional embryonic organs occurring during development (Wu, 2010). Although the
mechanisms that control heart development after the initial heart tube formation of fly are different from mammals, mechanisms that control the heart tube creation appear to be conserved in both fly and vertebrates. Xiushan Wu and his colleagues first identified the wingless (wg) as an important regulator in fly heart development (Wu et al., 1995; Park et al., 1996). Their subsequent studies further suggest the importance of Wg/Wnt signaling in fly heart development (Tang et al., 2013).

Dpp signaling is essential for patterning the wing. Jie Shen's group has explored the role of Dpp signaling on cell morphogenesis and proliferation control. The Dpp target genes optomotor-blind (omb) and spalt (sal) mainly mediate the Dpp's functions. Discontinuous in Omb and Sal activity lead to a re-organization of the epithelial cytoskeleton and basal retraction of cells (Tang et al., 2016). Sal activity promotes cell proliferation without regional manner in the wing disc (Wang et al., 2017). Omb regulates proliferation in a region-specific manner by control of the proliferation-promoting microRNA bantam (ban) expression. Dpp-Omb signaling inhibits proliferation in the medial wing disc by suppression of ban and promotes proliferation in the lateral wing disc by activation of ban (Zhang et al., 2013b). Omb orthologous human TBX2 (T-box transcription factor 2) and TBX3 are overexpressed in various cancers. Overexpressing Omb and TBX2 in the wing epithelium induces two types of cell motility, long distance migration within the plane of the epithelium and cell invasion across the basal lamina without degradation of extracellular matrix (Shen et al., 2014).

3.3. JNK signaling pathway

The JNK pathway plays crucial roles in animal development. Dysregulation of JNK signaling has been implicated in various types of human diseases including cancers, neurodegenerative diseases, immunity defects and metabolic syndromes. Lei Xue’s group identified a number components and modulators of JNK pathway. They showed that forkhead boxo (FoxO) is a downstream target of JNK, and the Pelle kinase can promote cell death by directly phophorylating FoxO (Wu et al., 2015). Moreover, they found that impaired Hippo signaling promotes Rho1-JNK-dependent growth (Ma et al., 2015b), and that the E3 ubiquitin ligase POSH regulates Hippo signaling through ubiquitin-mediated expanded degradation (Ma et al., 2018). On the other hand, they found that the Hippo pathway promotes JNK-dependent cell migration through miRNA ban-mediated Rux8 stability. Furthermore, Myc suppresses tumor invasion and cell migration by inhibiting JNK signaling (Ma et al., 2017).

3.4. Natural lineage reprogramming

Recently, a promising regenerative strategy has been suggested by converting a highly specialized cell into the desired cell identity in a living body. Although very rare, natural lineage reprogramming can occur with high efficiency and temporo-spatial precision. However, it is still unclear about the molecular mechanisms underlying in vivo lineage conversion. Yan Song's group has recently revealed a natural midgut-to-renale lineage reprogramming event during fruit fly metamorphosis. Moreover, they also identified a highly conserved protein called Cut as a crucial cell identity switch in this process. By intersecting with a pulse of steroid hormone, a steep gradient of a spatial molecule (Wingless/Wg) induces Cut to specifically express in a small subset of fly midgut progenitors and precursors that produce functional midgut cells, which are later converted into renal identity. These reprogrammed progenitors in turn migrate across organ boundary and onto renal tubules, and give rise to functional renal cells. Notably, although midgut progenitors depleted of Cut migrate normally onto renal tubules, they could not complete switching their cell identity and fail to give rise to midgut cells along renal tubules, causing renal identity crisis (Xu et al., 2018).

4. Metabolism and physiology

4.1. Metabolism and development

Drosophila shares similar metabolic pathways with mammals, and as an insect, it also has unique features in metabolism and physiology. Sheng Li’s group focuses on the functions and interplays of steroid and sesquiterpenoid hormones during developmental transitions. Their study revealed a crosstalk between these two hormones in the regulation of insect metamorphosis (Liu et al., 2018). Besides this, they also identified two distinct roles of the most abundant sesquiterpenoid, methyl farnesoate (MF). MF acts as a hormone directly through two juvenile hormone (JH) receptors, Met and Gce, and as the precursor of JH for maintaining larval juvenile status (Wen et al., 2015). Bing Zhou’s group studied iron homeostasis. They identified ZIP13 (also known as Slc39a13) as an iron transporter. Loss of function of ZIP13 results in multiple iron homeostasis defects, including iron decrease in the secretory compartments (Xiao et al., 2014).

Yong Q. Zhang’s group together with Zhaohui Wang’s group revealed developmental roles of Acyl-CoA synthetase ACSL4, which converts long-chain fatty acids to acyl-CoAs, in particular in synapse homeostasis. Drosophila ACSL4 mutants exhibit numerous defects in axonal transport, synapse development and function at the neuromuscular junction (NMJ) synapses (Zhang et al., 2009; Yao et al., 2011). Mechanistically, dACSL4 mutants show aberrant endosomal recycling and elevated BMP signal. Importantly, reducing the doses of the BMP pathway components can partially suppress the overgrowth of NMJ synapse (Liu et al., 2014b). Consistent with the enzymatic role of ACSL, dACSL4 affects the composition of fatty acids and membrane lipids; lipidomic analysis shows an elevated level of mannosyl glucosylceramide (MacCer) in dACSL4 mutant brain and genetic manipulations of MacCer support the close correlation of MacCer level with NMJ growth (Huang et al., 2016).

By studying genes in peroxisome biogenesis and sterol transport, Xun Huang’s group revealed roles of very long chain fatty acid (VLCP) and sterol in spermatogenesis. Peroxisome is important for the catabolism of VLCP. Several peroxisome biogenesis defective PEX (peroxins) mutants, where VLCPA was elevated, are male infertile. Notably, the cytokinesis defect is associated with the level of VLCP and lowing the level of VLCPA rescues the sterility of PEX mutants (Chen et al., 2010). In addition, their study revealed that Osbp (oxyester-binding protein) mutants with sterol transport defects exhibit individualization defects during spermatogenesis (Ma et al., 2010). However, how sterol homeostasis exactly affects spermatogenesis is not known.

4.2. Lipid metabolism

The eases of genetic manipulation and environmental intervention make Drosophila an excellent model for metabolic diseases such as obesity and diabetes. Xun Huang’s group focuses on studying lipid storage regulation in adipose tissue, the fat body, and non-adipose tissue, the salivary gland. By generating the first Drosophila model of the most severe lipid dystrophy disease BSCL2 (Berardinelli-Seip congenital lipodystrophy 2), they revealed distinct and autonomous functional mechanisms of Seipin, the gene mutated in human BSCL2, in both adipose and non-adipose tissues. Similar to mammalian BSCL2 models, Drosophila Seipin (dSeipin)
mutations exhibit opposite phenotypes in adipose and non-adipose tissues: dSeipin mutants have ectopic lipid droplet in salivary gland; while in fat body, lipid storage is greatly reduced in dSeipin mutants. They further demonstrated that elevated level of phos- phatic acid likely contributes to the ectopic lipid droplet pheno-
type in salivary gland (Tian et al., 2011). On the other hand, the reduced lipid storage phenotype in the fat body can be explained by the interaction of dSeipin and SERCA, a calcium ATPase in endo-
plasmic reticulum (ER), which regulates calcium level in the ER. ER calcium level is reduced in dSeipin mutant fat cells. Genetically restoring the ER calcium level rescues the lipodystrophy phenotype of dSeipin (Bi et al., 2014). Ultimately, the decreased ER calcium affects calcium level in mitochondria, where citrate, the precursor of lipogenesis, is produced through TCA cycle. Restoring mito-
chondrial calcium or replenishing citrate in the medium restores lipid storage in dSeipin mutants (Ding et al., 2018). Their study suggests that a dSeipin-SERCA mediated ER Ca\(^{2+}\)-mitochondrial Ca\(^{2+}\)-TCA axis contributes to BSCL2 lipodystrophy. Besides BSCL2 models, Xun Huang’s group also revealed other mechanisms in regulating lipid storage, including general transcription factor telomeric repeat binding factor 2 (TRF2)-mediated lipid droplet size regulation (Fan et al., 2017), CDF diglyceride synthetase (CdsA)-
involved lipid partition for storage and growth (Liu et al., 2014a), and the cooperation of two lipid droplet proteins perilipin 1 and 2 (PLIN1/PLIN2) in regulating the balance of lipid storage and lipoly-
ysis (Bi et al., 2012).

5. Neurological diseases

Wei Xie and Junhai Han’s groups used Drosophila as the model to investigate the roles of autism-related genes in neural development and neural transmission. They have demonstrated that the well-
known autism candidate genes encoding Neurexin and Neuro-
ligins predominantly express in pre- and post-synaptic terminals, respectively. Both Neurexin and Neureligins play critical roles in synaptogenesis. Loss of Neurexin and Neureligins results in reduced bouton numbers, aberrant presynaptic and postsynaptic development at Drosophila NMJ (Zeng et al., 2007; Sun et al., 2011). In addition, they have revealed that Neurexin restricts the axonal branches of visual L4 neuron in column through clustering of the classical axon guidance molecule, Ephrin (Liu et al., 2017b). Moreover, they have shown that Neurexin mediates retinoid transport through stabilizing the retinoid transporter apolipoproteins (Tian et al., 2013).

Except for neural development, they have demonstrated that Neurexin and Neureligins also play critical roles in synaptic trans-
mition. Wei Xie’s group has revealed that Neurexin regulates synaptic transmission in several steps. Firstly, Nurexirnx interacts with the Scribble-Pix complex to stimulate F-actin assembly and to promote synaptic vesicle clustering (Rui et al., 2017). Secondly, Neurexin interacts with N-ethylnmaleimide-sensitive factor (NSF) to regulate synaptic vesicle release (Li et al., 2015b). Thirdly, Neurexin functionally couples with the calcium channel Cac to regulate synaptic transmission. They also revealed that Neurelin 4 mod-
ulates gamma-aminobutyric acid (GABA) transmission in large ventral lateral neurons through recruiting GABAA receptors resis-
tant to dieldrin. Notably, even though Neurexin and Neurelin4 function in different brain regions, both of their mutant flies showed reduced night-time sleep (Li et al., 2013a). Additionally, Junhai Han’s group showed that GABAA receptor could be regulated by the ubiquitin E3 ligase FoxN4 (F-box and leucine rich repeat protein 4) to control clock output (Li et al., 2017b).

Mutations in Neurexins and Neureligins lead to autism, while loss of the ubiquitin ligase E3A (UBE3A) results in Angelman syn-
drome, which is characterized by severe mental retardation, developmental and speech impairment, ataxia, seizures, and happy disposition. Using the Drosophila NMJ as a model, Yong Q. Zhang’s group showed that the Drosophila homolog of UBE3A specifically targets Thickvein for ubiquitin-mediated degradation to down-
regulate BMP signaling at NMJ synapses (Li et al., 2016). Yong Q. Zhang’s group also showed that the fragile X mental retardation protein FMRP, loss of which results in the most common form of inherited intellectual disability Fragile X syndrome, is required for axonal transport of mitochondria and maintains genome stability (Yao et al., 2011; Liu et al., 2012; Jiang et al., 2016). Dynamical regulation of mitochondria (e.g., mitochondrial fusion, fission and transport) is involved in a variety of biological processes and regulated by a number of factors, including Miro. Lei Liu’s group found that the loss of vimar (atypical GEF coding gene) strengthened mitochondrial fission under normal physiological conditions. Their genetic analyses suggest that while loss of vimar rescues mitochondrial enlargement induced by a gain-of-function Miro transgene, a gain-of-function vimar transgene enhances Miro function. Thus, their findings identified a novel regulator of mito-
chondrial fission through Miro and indicated a potential ther-
apetical target for diseases in which mitochondrial fission and fusion are dysfunctional (Ding et al., 2016).

Long non-coding RNAs (lncRNAs) consist of a large proportion of the transcriptome among the species. Li Liu’s group identified a novel lncRNA, CASK regulatory gene (CRG), expressed in the nervous system of Drosophila, which could be involved in the regula-
tion of the movement in adult Drosophila, and this effect was modulated by the adjacent protein-coding gene, CASK (Ca\(^{2+}\)/calmodulin-dependent protein kinase). Moreover, they found that CRG is required for the recruitment of RNA polymerase II to the CASK promoter regions, which in turn enhances CASK expression. Their findings reveal new functional roles of lncRNAs and provide insights into understanding the pathogenesis of neurological dis-
ases associated with movement disorders (Li et al., 2012).

6. Learning, memory and decision-making

Fly fruit possessing a compact brain and complicated behaviors, serves an excellent model system for uncovering the nature of in-
telligence. Substantial achievements have been made in cognitive neuroscience field with this small insect, including information coding and processing, the neural circuit of learning and memory, the dynamics of memory, and most intriguingly, decision-making and remaking. Aike Guo’s group has made a serial of important contributions in this field. They demonstrated, for the first time, that flies make decisions based on conflicting learning cues (Tang and Guo, 2001), and the dopaminergic system plays a critical role in this advanced cognitive function (Zhang et al., 2007). They further investigated the reversal learning in both olfactory and vi-
sual systems, and this unique type of learning shares common circuit mechanisms with decision-remaking (Ren et al., 2012; Wu et al., 2012). Another interesting learning type studied in Aike Guo’ group is the cross-modal learning through the interaction between olfactory and visual cues (Guo and Guo, 2005) and his study illustrated potential neural pathway underlying this inter-
action (Zhang et al., 2013a).

Utilizing the optogenetics approach, Yan Li and Aike Guo’ groups together uncovered the novel function of gap junctions in visual learning of flying flies (Liu et al., 2016b) and revealed the neural circuit and molecular mechanisms underlying cross-modal learning (Zhang et al., 2013c); sleep (Yi et al., 2013), and addictive behaviors (Zhang et al., 2016). Yan Li’s group also characterized nutrient-specific feeding behavior and identified a protein-specific satiety factor (Liu et al., 2015; Sun et al., 2017). These findings allow in-depth investigation of feeding decision, as well as the neural
network that processes the decision-making.

7. Fly innate behaviors: aggression, courtship and sleep

Animals are born with the instinct to fight and mate. Individuals can still display innate aggression and courtship behavior even when isolated immediately after birth, suggesting that a genetic program specifies the neural circuits governing aggression and courtship. Yi Rao’s group has addressed neural mechanisms by which the neurotransmitter octopamine regulates aggression and courtship in *Drosophila*. Loss-of-function or gain-of-function of a gene encoding tyramine beta hydroxylase (TβH) required for synthesizing octopamine reduced or increased aggression, respectively, as did functional inhibition or activation of octopaminergic neurons. Interestingly, resupply of the TβH back to five octopaminergic neurons in the subesophageal region restored the aggression phenotype of flies lacking TβH, suggesting an important role for these five neurons in aggression (Zhou et al., 2008). In a related study, they examined the involvement of octopamine in courtship conditioning behavior, wherein male courtship is depressed by male experience with a previously mated female. They have revealed that octopamine regulates courtship conditioning through the octopamine receptor OAMB, which is preferentially expressed in the mushroom body, the primary site for fly memory (Zhou et al., 2012). These findings have contributed to a mechanistic understanding of how neural circuits are regulated by neuromodulators to generate adaptive behaviors in different social contexts.

Sleep homeostasis is essential from flies to humans. But the molecular mechanisms underlying sleep homeostasis are poorly understood. Yi Rao’s group have characterized the roles of neurotransmitter 5-hydroxytryptamine (5-HT) in *Drosophila* sleep (Qian et al., 2017). They found that Trh (thyrotropin releasing hormone) and ShH2b (5-HT receptor 2B) knock-out mutants displayed decreased sleep time and diminished sleep rebound after sleep deprivation. They also revealed the expression of ShH2b in a small subset of dorsal fan-shaped body (dFB) neurons is essential for sleep homeostasis. Genetic ablation of ShH2b neurons in the dFB led to the decreased sleep and impaired sleep homeostasis. Their results suggest that serotonergic signaling in specific neurons is required for the regulation of sleep homeostasis.

Besides, some endocrine hormones and neuropeptides are also important for the regulation of sleep and sleep homeostasis. Zhangwu Zhao’s group characteristic that sleep deprivation increases transcription of short neuropeptide F (sNPF) and wakefulness at night in control flies but not in the sNPF mutant flies, suggesting that sNPF autoregulation plays an important role in sleep homeostasis. They also showed that sNPF could modulate sleep through the sNPF-cAMP-PKA-CREB signal pathway in vivo, and subsequently activates the downstream CREB transcription factor (Chen et al., 2013). Moreover, they found that JH signal pathway is critical for maintenance of sexually dimorphic sleep by regulating sex-relevant genes, in which the sexually dimorphic sleep induced by JH signals is a change of sleep drive and independent of the circadian clock (Wu et al., 2018).

8. Epigenetic regulation

8.1. Posttranscriptional regulation

The core of developmental biology is to understand how the developmental signals are controlled in a spatial and temporal manner to guide cell fate decisions, tissue patterning and adult homeostasis. Currently, most research focuses on the identification of transcriptional targets and consequences of developmental signal transduction. However, very little is known regarding the molecular and cellular mechanisms of how developmental signals are produced, received and interpreted. Jian Zhu’s group utilizes the *Drosophila* wing morphogenesis as a model system to understand how developmental signals are initiated and transduced across the cell membrane. They uncovered an epigenetic control mechanism for Notch signal production (Du et al., 2016), revealed a crucial role of the RNA regulatory machinery and cell polarity determinants in cell reception of the Wingless signal (Liu et al., 2016a), and finally, developed a model by which cells utilize protein posttranslational modifications to convert differential concentration gradient of Hh morphogen into distinct developmental outcomes (Su et al., 2011; Zhang et al., 2014). These original findings not only increase our understanding of molecular networks on how cells produce, recognize and interpret developmental signals, but also establish a causal link between misregulation of developmental signaling and diseases.

A hallmark of aging in mammals is the occurrence of age-dependent ectopic fat accumulation (EFA), which contributes to age-related tissue deterioration and dysfunction. By using a fly model, Renjie Jiao’s group assessed the molecular basis of age-dependent EFA formation (Yan et al., 2017). They characterized the occurrence of age-dependent EFA in *Drosophila* and identified HDAC6, a cytosolic histone deacetylase, as a suppressor of EFA. Their study suggests that, in an age-dependent manner, EFA mainly appears in the thoracic jump muscles of adult flies. Further, they identified the suppressors of age-dependent EFA, dHDAC6 and dHsc4 (*Drosophila* heat shock cognate gene 4) which are proteostatic regulators. The genetic and biochemical analyses indicated that dHDAC6 maintains the proteostasis of lipid droplet protein PLIN2 by regulating the acetylation level of dHsc4. The dHDAC6-dHsc4-PLIN2 axis links proteostasis to fat metabolism during aging. Together, their results point out that it is the protein quality rather than the protein quantity of PLIN2 that controls age-dependent EFA.

8.2. piRNA

Small noncoding RNAs (ncRNAs) including miRNAs, siRNAs and piRNAs play important roles in regulating gene expression through a variety of mechanisms. P-element induced wimpy testis (PIWI)-interacting RNAs (piRNAs), a subset of small ncRNAs (~24–30 nt in length), associate with PIWI proteins and play conserved roles in silencing transposons, and thus maintaining genome integrity and germline development. Previous studies suggested two models for piRNA production, including the primary processing and the feedforward amplification pathways (Brennecke et al., 2007; Gunawardane et al., 2007). Dahua Chen’ group investigated the role of argonaute 3 (AGO3) Slicer activity in regulating piRNA biogenesis. They showed that AGO3 inhibits the homotypic Aubergine (Aub:Aub) Ping-Pong process and the expression of an AGO3 Slicer mutant causes ectopic accumulation of Armitage. AGO3 also coexists and interacts with Armitage in the mitochondrial fraction. Moreover, they found that AGO3 acts in conjunction with Zucchini to control the dynamic subcellular localization of Armitage between mitochondria and nuage. Thus, these findings uncover a new mechanism that regulates secondary piRNA amplification (Huang et al., 2014). Rongwen Xi’s group found that *Pelo* (Dm34)-Hbs1 mRNA surveillance complex is required for transposon silencing, but not required for piRNA biogenesis in the *Drosophila* germline. Their results suggest that Pelo may silence transposable elements at the translational level (Yang et al., 2015).

8.3. Discovery of a novel DNA modification

N6-methyladenine (6mA) is one of the most abundant types of DNA methylation, and plays epigenetic roles in regulating a number of processes.
of biological processes in bacteria (Wion and Casadesus, 2006). However, the role of 6mA in higher eukaryotes has long been a mystery. Using Drosophila as a model, Dahua Chen’s group recently showed that 6mA is present in the Drosophila DNA at a relatively high level at the very earliest embryonic stages, but at low levels at the late embryonic stages. Moreover, they found that the dynamic modification of 6mA in early embryos is actively regulated by the Drosophila DMAD (DNA N6-methyladenine demethylase) protein, a homolog of Ten-eleven translocation (Tet) protein. Using the DMAD mutant flies, they found that DMAD is essential for Drosophila development and suppresses adenine methylation in genome. DMAD has no apparent in vivo role in regulating the conversion of 5mc to 5hmC, but can directly catalyze 6mA demethylation in vivo and in vitro. Using high-throughput sequencing analysis, they found that the expression levels of these 6mA peaks related transposons in DMAD mutant ovary sample were significantly higher than those in wild type. Taken together, their findings suggest that DMAD-mediated 6mA mode methylation is correlated with transposon expression (Zhang et al., 2015).

9. Future of the fruit fly research

Drosophila has made significant contributions to scientific research, and in the future, it undoubtedly holds a great promise to help biologists to investigate the mysterious fundamental question in many aspects. For example, germ cells are the only type of cells that can pass the genetic materials from one generation to the next. One of the most fundamental questions in life science is how cells that can pass the genetic materials from one generation to the other. For example, it has been recently revealed that the genetic and molecular regulation of sleep: from fruit fly to humans. Nat. Rev. Neurosci. 10, 549–557.

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