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REVIEW

Drosophila Studies on Autism Spectrum Disorders

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Abstract In the past decade, numerous genes associated with autism spectrum disorders (ASDs) have been identified. These genes encode key regulators of synaptogenesis, synaptic function, and synaptic plasticity. *Drosophila* is a prominent model system for ASD studies to define novel genes linked to ASDs and decipher their molecular roles in synaptogenesis, synaptic function, synaptic plasticity, and neural circuit assembly and consolidation. Here, we review *Drosophila* studies on ASD genes that regulate synaptogenesis, synaptic function, and synaptic plasticity through modulating chromatin remodeling, transcription, protein synthesis and degradation, cytoskeleton dynamics, and synaptic scaffolding.

Keywords Autism spectrum disorders · *Drosophila* · Chromatin remodeling · Synaptic scaffolding · Synaptic transmission

Introduction

Autism spectrum disorders (ASDs) are complex developmental disabilities, whose prevalence is estimated to be 1 in 68 children under 8 years of age in the USA, and they differ substantially between boys (1 in 42) and girls (1 in 189) [1]. The core diagnostic features are impaired social interaction, and repetitive and restrictive behaviors [2]. In addition, children with ASDs frequently present with a host of associated behavioral issues, such as motor deficits (hypotonia, apraxia, or motor delay), sleep abnormalities, gastrointestinal disturbances, and epilepsy [3–7]. In the past decade, numerous mutations in ASD-associated genes have been identified and various mouse models of monogenic forms of ASDs have been generated and characterized [8]. So far, a variety of standard assessments for ASD-related behavioral phenotypes have been established in mouse models [9]. Meanwhile, ASD models in other animals such as nonhuman primates and *Drosophila* are necessary complements in ASD studies, and are valuable in translating genetic findings and deciphering the shared molecular pathways and phenotypes in ASDs [8, 10].

The fruit fly *Drosophila melanogaster* is a prominent model system in neuroscience. As a model system, it has a wide range of practical and genetic advantages, such as a short generation time ($\sim$10 days at room temperature) and a large number of offspring for rapid large-scale analysis (females can lay up to 100 eggs per day). In addition, *Drosophila* has some unique aspects for genetic studies, including the lack of meiotic recombination in males and the use of balancer chromosomes that carry visible genetic markers to facilitate the maintenance of mutant lines [10]. *Drosophila* is also useful for defining gene interaction networks and identifying novel regulatory connections. It offers efficient and high-throughput genetic manipulation, and greatly facilitates the discovery of single gene functions, neurogenetic events, and advanced behaviors [10, 11]. Despite the low anatomical conservation, the biological processes are highly conserved between *Drosophila* and humans at the molecular, cellular, and synaptic levels. About 75% of human disease genes have identifiable homologs in *Drosophila*, 44% of which are sufficiently conserved for functional study [12, 13]. Here, we review the studies in *Drosophila* that characterize the

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genetic and molecular pathology of ASDs. These studies involve many ASD-associated genes that influence the structure and the turnover of synapses at different levels, including chromatin remodeling, transcription, protein synthesis and degradation, actin cytoskeleton dynamics, and synaptic transmission (Fig. 1).

Chromatin Remodeling and Transcription

Some important regulators of chromatin remodeling and transcription are promising genetic factors for ASDs. However, how changes in these genes affect neuronal morphology and activity is unclear. Several studies in *Drosophila* have revealed the underlying molecular mechanisms of chromatin remodeling and transcription regulators in neural development and ASD-related behaviors (Figs. 1, 2).

Mutations in *POGZ* have been reported in individuals with ASDs, intellectual disability, and schizophrenia [14–16]. *POGZ* encodes a heterochromatin protein 1 α-binding protein and is hypothesized to function as a transcriptional regulator in molecular networks crucial for neuronal function [17]. Downregulation of *row* (*Drosophila* ortholog of *POGZ*) in neurons leads to deficits in habituation, a form of non-associative learning that is relevant to both intellectual disability and ASDs [18]. Euchromatin histone methyltransferase (*EHMT*) is another ASD risk gene, which encodes a member of the evolutionarily-conserved protein family methylates histone 3 at lysine 9 [19–21]. In *Drosophila*, loss of Ehmt results in a significant decrease of dendrite end number, higher-order branching, and dendritic field complexity, as well as learning and memory deficits [22].

Disrupted-in-schizophrenia-1 (*DISC1*) is associated with a wide range of mental illnesses, including ASDs [23]. DISC1 interacts with and activates transcription factor 4 (ATF4)/CREB2 in the nucleus [24]. A fly model expressing human *DISC1* has shown that accumulation of exogenous human DISC1 in the nucleus disturbs sleep homeostasis, implying a deficit in neuronal activity. This function is modulated by interaction with ATF4/CREB2 and recruitment of a co-repressor, N-CoR, to the CRE-mediated transcriptional machinery [25].

MicroRNA (miRNA) is another way to post-transcriptionally regulate gene expression. The autism susceptibility gene *A2bp1* has been identified in *Drosophila* as an mRNA target of miR-980 [26]. MiR-980 inhibition enhances olfactory learning and memory stability, while its overexpression in the mushroom bodies impairs 3-h memory. Overexpression of its target *A2bp1* in the mushroom bodies enhances memory. These defects may be attributed to the role of miR-980 in inhibiting excitability, as projection neurons overexpressing miR-980 exhibit a strong trend for a lower mean firing frequency with an injected current at 40–50 pA[26].

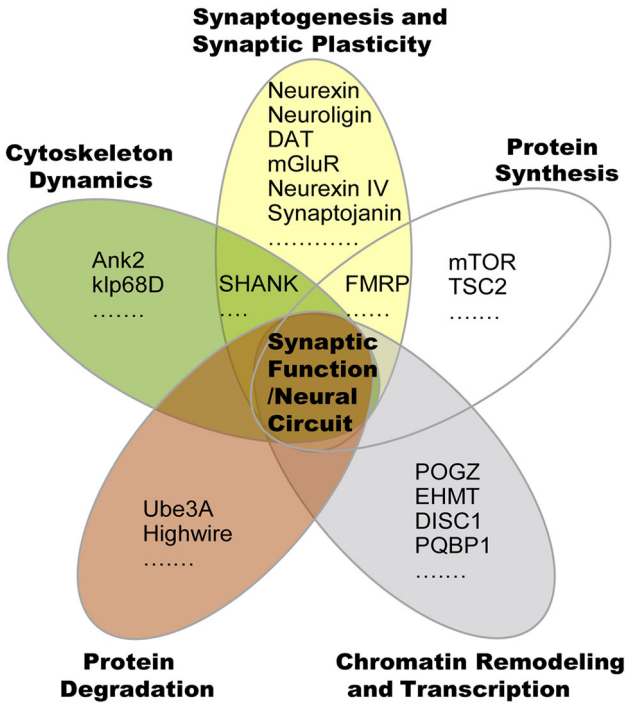

Fig. 1 ASD-associated genes regulate synaptic function and neural circuits through various cellular events.

Protein Synthesis and Degradation

Neuronal activity and function are partially determined by synaptic protein levels, which are strictly regulated by protein synthesis and degradation. On the other hand, the levels of synaptic proteins are also influenced by neuronal activity [27]. Mutations of the genes involved in such homeostatic regulation have been found in ASD patients [28]. Numerous studies in *Drosophila* have illustrated that dysfunction of ASD-related genes affects protein synthesis and degradation, and subsequently results in deficits in synaptogenesis and synaptic function, as well as synaptic plasticity (Fig. 2).

The fragile X mental retardation 1 gene (*FMR1*) encodes a pan-neuronal RNA-binding protein, FMR1, that associates with specific mRNAs to repress their translation [29–33]. So far, >800 distinct mRNA targets of FMR1 have been found, and it has been implicated in many aspects of brain development and function [34]. Fragile X Syndrome (FXS), the leading monogenetic cause of autism, is caused by transcriptional silencing of the *FMR1* gene due to a trinucleotide repeat expansion in its 5'-UTR [35, 36]. Since the generation of the first *Fmr1*-knockout

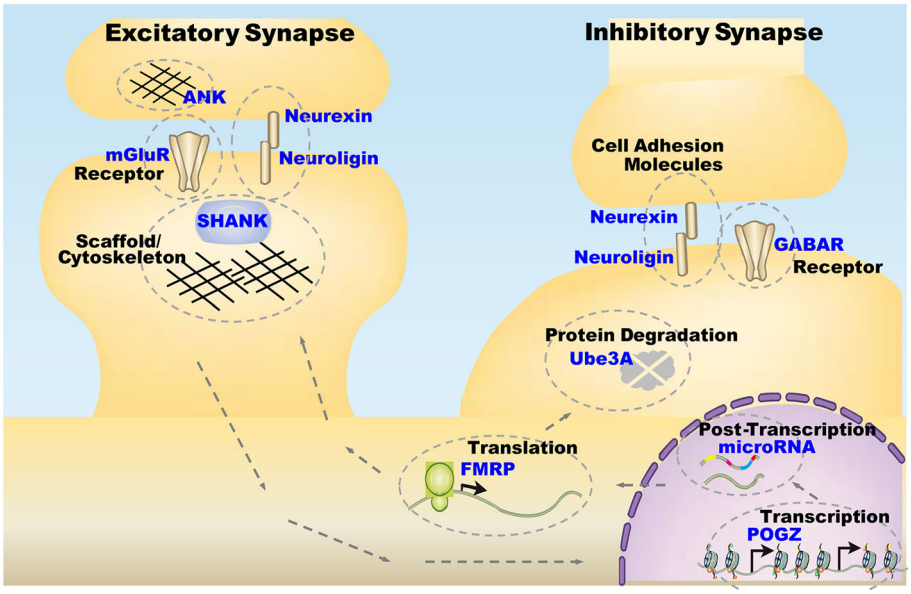

Fig. 2 Functions of ASD-associated genes in different cellular processes.

mouse model to study FXS, other animal models including the fly FXS model have been further developed and studied, providing more cellular and molecular clues to explain this complex syndrome. There is only one *FMR1* homolog, named *dfmr1* in *Drosophila*, which encodes the dFmr1 protein that shares high identity with mammalian FMR1 in the functional domains [37]. The *Drosophila* neuromuscular junction (NMJ) is a glutamatergic synapse characterized by stereotypic innervation patterns of motor neurons into well-defined target body-wall muscles, making it easier to study synaptogenesis, synaptic transmission, and plasticity [38]. *Drosophila dfmr1* loss-of-function mutants show synapse overelaboration (overgrowth, overbranching, and excess synaptic boutons) in peripheral NMJs [39] as well as in the mushroom body (MB) of the central nervous system [40], accompanied by altered neurotransmission. The hypermorph mutants of *dfmr1* show opposite defects. A further rescue study indicated a pre-synaptic requirement of dFMR1 for synapse structuring, along with both a pre- and post-synaptic requirement for functional neurotransmission [41]. Furthermore, *dfmr1* loss-of-function mutants exhibit more dendritic branching in dendritic arborization neurons and its role in dendrite development is partially mediated by Rac1 as well as microRNA-124a [42, 43]. In addition, deficits in axonal targeting have been extensively reported in *dfmr1*mutants [40, 44–47].

Loss of Fmr1 up-regulates the translation of its target mRNAs [39, 42, 48, 49]. In the fly NMJ, *Adar* acts downstream of *Fmr1* for proper NMJ architecture [50]. The other two dFmr1 targets, the synaptic heparan sulfate proteoglycans glycosylphosphatidyl inositol-anchored Dally-like protein (*Dlp*) and transmembrane Syndecan (*Sdc*), play important roles in modulating synaptic structure and function [51]. The expression of these two proteins is markedly elevated in *dfmr1*-null NMJs [52]. Bone morphogenetic protein type II receptor (*BMPR2*) is also one of the targets of FMR1. The structural defects at the NMJ in loss-of-function *dfmr1* mutants can be rescued by reducing *Wit*, the *Drosophila* ortholog of *BMPR2* [53]. The role of FMR1 in mRNA translation is regulated by polyglutamine-binding protein 1 (PQBP1), whose mutations cause Renpenning syndrome [54]. *Drosophila* Pqbp1 interacts with Fmr1 and facilitates target mRNA assembly into ribosomes [54]. Two of the common mutations found in Renpenning syndrome, PQBP1 c.459_462delAGAG and c.463_464dupAG, encode a distinct C-terminal epitope that preferentially binds non-phosphorylated FMRP and promotes its ubiquitin-mediated degradation [55]. Therefore, PQBP1 c.463_464dupAG transgenic flies show remarkable defects of synaptic over-growth similar to *dfmr1* mutants, which can be rescued by exogenously-expressed dFmr1 [55].

The mTOR pathway controls global mRNA translation and has been shown to play roles in many fundamental cellular processes including autophagy, transcription, and cytoskeletal dynamics [56]. It is a key regulator of neuronal differentiation [57, 58], and hyper-activation of this pathway increases the risk of ASDs [59]. In *Drosophila*, the gene *unkempt* (*unk*) has been identified as a novel negative regulator of photoreceptor differentiation acting downstream of mTORC1. Unk together with its binding partner headcase (*Hdc*) negatively regulates the InR/mTOR pathway and controls the timing of neurogenesis [60, 61]. Tuberous sclerosis complex (TSC) is an autosomal dominant disorder that shows clinical features of epilepsy and

autism. It is caused by mutations in the *TSC1* or *TSC2* genes. TSC protein regulates synaptic growth *via* the TORC2-Akt pathway, and the mTOR pathway is upregulated by TSC2 protein[59]. In the *Drosophila* NMJ, *Tsc2* mutants show increased synaptic growth [62].

Duplications of the genomic region encompassing *UBE3A* (15q11–q13) are the second most common genetic lesions found in autism (3%–5% of cases) [63]. *UBE3A* is a maternally-expressed gene and its product, the ubiquitin protein ligase E3A, is present in many regions of the brain, with highest expression in the hippocampus and cerebellum [64]. In *Drosophila*, loss of Ube3a significantly increases the number of both total and satellite boutons in conjunction with compromised endocytosis in the NMJs [65]. Ube3a specifically ubiquitinates the type I BMP receptor Tkv, and promotes its proteasomal degradation. Therefore Ube3a has a critical role in regulating NMJ development by repressing BMP signaling [65]. Over-expression of *Ube3a* in *Drosophila* results in a decreased number of active zones per bouton and vesicle area, and 40%–50% of larvae intermittently fail to evoke junction potentials at rapid stimulation rates (15 Hz) [66]. Both loss-of-function and over-expression of Ube3a decrease dendritic branching, suggesting that the proper level of Ube3a is critically important for normal dendritic patterning [67]. Moreover, improper expression of *Ube3a* appears to be detrimental to learning, thereby recapitulating the learning deficits in autism [68]. Over-expression of human *Ube3a* in *Drosophila* that mimics the gene duplication in autism patients has been used to screen UBE3A substrates [69]. A key regulator of monoamine synthesis, the gene *Punch* or *GCH1* encodes the enzyme GTP cyclohydrolase I [70]. It is interesting that the mRNA and protein levels of the *Drosophila* vesicular monoamine transporter dVMAT are also elevated in the absence of dFmr1 [71]. The altered monoamine (dopamine/serotonin) synthesis pathway found both in *dUBE3A* and *dFMR1* mutants may provide a potential explanation for the repetitive behaviors and hyperactivity associated with autism and also explain why some individuals with ASDs respond better to selective serotonin reuptake inhibitors than others.

Highwire is another highly-conserved E3 ligase associated with ASDs, and it functions presynaptically to negatively regulate synaptic growth at the *Drosophila* NMJ [72]. Mutations of *wallenda*, which encodes an MAP kinase kinase kinase homologous to the vertebrate dual leucine zipper-bearing kinases *DLK* and *LZK*, completely suppress the synaptic overgrowth phenotype in *highwire* mutants [73]. In addition, Rae1 has been identified as a Highwire cofactor to prevent the autophagy-mediated degradation of Highwire protein in post-mitotic neurons [74].

Cell Adhesion Molecules

ASDs also involve many proteins mediating neuronal connectivity and synaptic transmission, such as the synaptic adhesion molecules neurexin (*NRX*) and neuroligin (*NLG*) in synaptogenesis, various neurotransmitters and proteins associated with synaptic vesicle recycling in synaptic transmission, contactin-associated proteinlike 2 (*CNTNAP2*) in neuronal conduction, and Ca^{2+} channels in ion permeability (Fig. 1). Given that the *Drosophila* genome is relatively less redundant than the human genome, a single mutation in the homolog of an ASD-related gene is more likely to avoid compensatory effects and yield a measurable phenotype.

The well-known autism candidate genes *NRX* and *NLG* are classical trans-synaptic partners that play critical roles in synaptogenesis and synaptic transmission (Fig. 2). Loss of Nlgs and Nrx results in reduced bouton numbers, aberrant presynaptic and postsynaptic development at NMJs, and impaired synaptic transmission [75–79]. It has been found that *Drosophila* neuroligin 1 (dNlg1) and dNlg3 act predominantly in pre-synaptic terminals, while dNlg2 functions both pre- and post-synaptically [75–78]. Nrx and Nlgs also play critical roles in synaptic transmission. dNrx has been shown to functionally couple with Ca^{2+} channels to regulate synaptic transmission. And dNlg4 modulates GABA transmission in large ventral lateral neurons through recruiting $GABA_A$ receptors resistance to dieldrin [80]. Interestingly, both *dnrx* mutants and *dnlg4* mutants exhibit reduced night-time sleep, even though they function in different brain regions [80, 81]. In addition, impaired dNrx and dNlgs result in neuronal plasticity defects [80, 82]. dNrx has been shown to interact with N-ethylmaleimide-sensitive factor (NSF), an enzyme that mediates disassembly of the soluble NSF attachment protein receptor (SNARE) complex, and plays an important role in synaptic vesicle release [83]. Besides, dNrx is expressed beginning from an early neurodevelopmental stage prior to synaptogenesis. dNrx plays an essential role in columnar restriction during L4 axon branching in the *Drosophila* visual system through clustering of one of the classical axon guidance molecules, Ephrin, which implies a novel role of dNrx in early neural development [84].

Neurexin IV (*NrxIV*) is an ortholog of the autism gene *CNTNAP2* [85]. *NrxIV* homozygous-null mutants display reduced bouton numbers, while heterozygous-nulls do not observably differ from the wild-type [86]. The polygenic causes of ASDs have also been investigated in *Drosophila* models using orthologs to human ASD genes with different copy number variants (CNVs) [87]. Two ASD candidate genes encoding adherens junction proteins, *NOTCH1* and *p120ctn* (orthologs of human *NOTCH1* and catenin delta 2 (*CTNND2*)) that show gain or loss of CNVs, respectively,

were tested pairwise and have shown synergistic effects on NMJ bouton number [87]. These studies provide evidence for synergistic interactions between CNV candidate gene sets, supporting shared and distinct genetic etiologies of ASDs.

Synaptic Receptors and Ion Channels

The *Drosophila* NMJ is an asymmetric glutamatergic synapse formed between motor neurons and muscle cells. Since it displays some advantageous features, including structural accessibility, stereotypic features, and amenability to genetic manipulations as well as electrophysiological and microscopic analyses, it is considered to be a convenient and useful model for elucidating the mechanisms underlying synapse formation, synaptic transmission and plasticity, and synaptic degeneration. Many mutants of genes encoding the components in synaptic transmission are also associated with ASDs, including synaptic receptors, components in synaptic vesicle cycling, and ion channels (Figs. 1, 2).

Mutations in the *Drosophila* group II metabolic glutamate receptor gene (*DmGluRA*) increase neuronal excitability by preventing PI3 kinase activation and consequently hyper-activating the transcription factor *Foxo* [88]. Loss of dFmr1 also results in excessive activity of metabotropic glutamate receptors (mGluRs) and learning and memory deficits, which are related to the inhibition of cAMP signaling reported in patients and animal models [36]. These deficits can be rescued by pharmacological inhibition of mGluRs, which provides further support for the agonistic effects of dFmr1 and mGluR signaling [89].

Several genes involved in the dopamine (DA) network are also associated with ASDs, including the plasma membrane protein syntaxin 1 (*STX1*) [90] and the DA transporter (*DAT*) [91]. A novel *de novo* missense mutation in the human DAT (*hDAT*) gene results in a Thr-to-Met substitution at site 356 (hDAT-T356M). Expression of hDAT-T356M in DA neurons with the *Drosophila DAT*-null allele leads to hyperlocomotion, indicating that alterations in DA homeostasis may confer risks for ASDs and related neuropsychiatric conditions [91]. Another two missense variants, *SLC6A3 R/W* and *STX1A R/Q* [17, 92, 93], disrupt the reverse transport of DA, resulting in DA dysfunction and associated locomotor behavioral abnormalities [94].

Acetylcholine is the major excitatory neurotransmitter in the central nervous system of insects [95]. The α7 subunit of the nicotinic acetylcholine receptor is one of the most prevalent receptors that are involved in neurological pathologies including autism [96]. In *Drosophila*, Dα7 protein is enriched at the dendrites of the giant fiber that integrates sensory input, activates flight motor neurons, and mediates synaptic transmission [97].

To date, three GABA receptor subunit classes have been cloned in *Drosophila.* They are *Rdl* (resistant to dieldrin), *Grd* (GABA and glycine-like receptor of *Drosophila*) and *Lcch3* (ligand-gated Cl^- channel homolog 3) [98]. A significant reduction has been found in all three subunits and glutamic acid decarboxylase in *dfmr1* mutants [99, 100].

Normal synaptic vesicle recycling contributes to functional neurotransmission. Synaptojanin (Synj) is a phosphoinositide phosphatase known to play an important role in synaptic vesicle recycling [101]. Dyrk1A, also known as Minibrain (Mnb), is a serine/threonine kinase implicated in ASDs [15, 102]. The protein encoded by the *Drosophila Mnb* gene has been shown to interact with the *INI1* ortholog *Snr1*, which is a chromatin-remodeling factor involved in the morphogenesis of dendritic arbors in *Drosophila* sensory neurons [103, 104]. Phosphorylation of Synj by Mnb kinase enhances Synj activity and is required for reliable synaptic vesicle recycling [105]. Hence, it is not surprising that *Synj* and *Mnb* mutations have been linked to autism [15, 102].

The *α2δ* gene family plays roles in Ca^{2+} channel trafficking and membrane stabilization-dependent synaptic morphogenesis [106], and is associated with a wide range of neurological diseases, including ASDs [17]. Presynaptic homeostatic potentiation is disturbed when *α2δ-3* is lost, due to a failure to potentiate presynaptic Ca^{2+} influx and the Rab-3 interacting molecule-dependent readily-releasable vesicle pool [107].

Scaffolding Proteins and the Actin Cytoskeleton

The correct positioning of cell-adhesion molecules, receptors, and channels at the synapse requires the complex assembly of scaffolding proteins and the actin cytoskeleton. Many mutations in these genes are also found in ASD patients (Figs. 1, 2).

The *SHANK* family gene *SHANK3* is considered to be one of the most prevalent causes of ASDs [108, 109]. Prosap/Shank family proteins have multi-domains including ankyrin repeats, SH3, PDZ, proline-rich, and SAM domains, and are the key organizers of the postsynaptic density (PSD) [110]. One possible molecular pathogenesis is an imbalance between excitatory and inhibitory receptors linked with the Nlgs-PSD-95-SHANK complex *via* PDZ binding. In mammals, the Shank family binds Nlgs and functions to coordinate pre/postsynaptic signaling through Neurexin-Neuroligin signaling complexes [111, 112]. There are three *Shank* family genes in mammals but only a single homolog of *Shank* in *Drosophila*, which makes it easier to implement *in vivo* null-mutant studies in *Drosophila* [113]. Both loss and over-expression of *Shank* decrease synaptic bouton number and maturity, and result in defects in the organization of the sub-

synaptic reticulum, a complex system of folding the postsynaptic membrane at the NMJ. Furthermore, Shank regulates a non-canonical Wnt signaling pathway in postsynaptic cells by modulating the internalization of the Wnt receptor Fz2 [114]. These findings imply that *Shank* dosage is critical for synaptic development and establish a novel connection between Shank and synaptic Wnt signaling. The Neurobeachin (*NBEA*) gene has been shown to be disrupted in patients with idiopathic autism [115]. *NBEA* encodes a neuron-specific multi-domain signal scaffold protein that is predominantly expressed in the brain during development [116]. Loss of Rugose (*rg*), the *Drosophila* homolog of human *NBEA*, results in abnormal synaptic architecture and physiology [117].

Cytoskeleton dysregulation is one of the major problems in *Fmr1* mutant neurons. The microtubule (MT) network is apparently altered in both loss- and gain-of-function *dfmr1* mutants [48, 118]. The number and transport of mitochondria in axons are also affected by dFmr1 [118]. Besides, live imaging shows that dFmr1-associated mRNA granules are less motile and show decreased directional movement in cultured *dfmr1* mutant neurons, demonstrating that FMR1 indeed regulates the association between mRNA cargos and microtubules [119]. *Drosophila* Fmr1 targets like *Futsch* and *Profilin* regulate MT- or actin-dependent synaptic growth and function [39, 49]. *Drosophila Ank2* is the closest homolog of human *ANK2* and *ANK3*. It is expressed specifically in the nervous system and associates with the presynaptic membrane cytoskeleton, similar to the human autism gene *ANK3* [120]. Ank2 functions downstream of Spectrin in the anchorage of synaptic microtubules. As a consequence, synaptic stability is severely disrupted in *Ank2* mutants, resulting in a reduction in overall terminal size, withdrawal of synaptic boutons, and disassembly of presynaptic active zones [120, 121]. Knock-down of *Ank2* in mushroom bodies shows normal learning but a significant reduction in short-term memory, suggesting a specific role of *Ank2* in cognition [122]. CNVs at 16p11.2 have recently been implicated in the pathogenesis of ASDs [123], but the genes responsible for the increased risk of ASDs are currently unknown. A Kinesin-2-encoding gene *klp68D* closely related to human *KIF22* at the 16p11.2 locus has been identified by genetic screening using the *Drosophila* NMJ system. Disruption of *klp68D* induces ectopic targeting of motor axons [124], suggesting that Kinesin proteins are important for synaptic connectivity.

Others

Children born to older parents are at a higher risk for disorders such as schizophrenia and autism. Studies in *Drosophila* models have provided evidence for parental age-related memory impairment [125]. Bisphenol A (BPA), a widely-used chemical in plastic containers, has been suggested to play a role in developmental disorders including autism [126]. Flies exposed to BPA show many autistic-like behaviors, which emphasizes the importance of environment etiology for neurodevelopmental disorders such as autism.

Conclusions and Perspective

In the past decade, ASDs have undergone considerable diagnostic evolution. To discover the novel biomarkers and molecular pathology underlying these disorders, several animal models have made contributions. While fruit-flies and humans have very different body plans, *Drosophila* has been a prominent model system in neuroscience since the 1960s for the remarkable similarity at the biological process level, such as a similar origin of the central nervous system [127] and several similar neurobiological processes including membrane excitability, neuronal signaling, and classes of neurotransmitters [128]. Several landmark studies have made discoveries ranging from single genes and neurogenetic events to advanced behaviors. Neuroscientists have taken advantage of *Drosophila* for its relatively simple nervous system, a powerful genetic toolkit, high throughput, and low cost. One of the best successes in *Drosophila* is the Fragile X story, reviewed above. In this case, the fly model can phenocopy FXS patients and FMR1-knockout mice, while pharmacological tests have been used to develop a potential treatment. Moreover, taking advantage of high-throughput screening, several Fmr1 targets have been screened in *Drosophila*, which help to explain the pathogenesis of FXS.

However, with the increasing thorough research on ASDs and the use of primates in ASD studies, what can be further contributed by research in *Drosophila*? Growing evidence supports a causal role for combinatorial contributions of multiple loci in ASD pathogenesis. Thus, it is necessary to investigate the roles of multiple gene interactions in ASD cases. As a good *in vivo* model with a powerful genetic toolkit, accompanied by the development of informatics-targeted screening, *Drosophila* will definitely show its power in identifying multiple candidate interactions, revealing distinct molecular etiologies underlying ASDs.

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