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RESEARCH HIGHLIGHT

A New Link Between Insulin Signaling and Fragile X Syndrome

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Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability and the most common known genetic cause of autism or autism spectrum disorders. FXS is caused by silencing or mutation of the fragile X mental retardation gene (*FMR1*), a known RNA-binding protein that acts as a negative regulator of translation [1, 2]. FXS patients demonstrate a myriad of symptoms that can vary widely between individuals, including impaired cognition, physical abnormalities, sleep problems, hyperarousal to sensory stimuli, increased anxiety, obsessive compulsive disorder-like behavior, attention-deficit hyperactive disorder symptoms, self-injurious behavior, aggression, and increased risk of seizures [3]. The molecular mechanisms underlying FXS are not clear, and currently there is no ideal treatment.

The *Drosophila* homolog of mammalian FMR1, dFMR1, contains several highly conserved domains that are also found in human FMR1 [1]. Loss of dFMR1 or expressing a mutant that carries deletions or point mutations in the coding region can lead to several behavioral and anatomical defects in the fly, including defects in learning and memory, social interaction, circadian rhythm, and sleep [2, 4–7], many of which are similar to those seen in FXS patients. The conservation of FMR1 protein between *Drosophila* and human, as well as the similarity in phenotypes when the gene is disrupted makes it possible to use fruit fly, this powerful, fast, and cost-effective genetic model, to investigate the molecular mechanism underlying FXS and facilitate drug development.

In a recent paper published in Molecular Psychiatry [8], researchers from the University of Pennsylvania uncovered misregulated insulin signaling (IS), which contributes to circadian and memory deficits in the *Drosophila* FXS model. Expression of d*fmr1* in the insulin-producing cells of the brain is sufficient to rescue the memory deficits and restore normal circadian behavior in d*fmr1* mutant flies. Moreover, the protein level of insulin-like peptide DILP2 is elevated in d*fmr1* mutant flies. What is the consequence of increased DILP2 in the brain? A GFP-pleckstrin homology (PH) domain reporter was used by the authors to monitor the activation of phosphoinositide 3-kinase (PI3K), and increased membrane localization of the reporter was detected in d*fmr1* mutant brains, indicating stronger PI3K activation. As PI3K is known to phosphorylate Akt at S505, the authors next examined phosphorylation at this particular site. Consistently, an enhanced p-S505-Akt signal was detected at the plasma membrane of d*fmr1* mutants, further validating elevated PI3K signaling in these flies. Moreover, rescuing d*fmr1* significantly decreased DILP2, membrane GFP-PH, and p-S505-Akt levels, indicating that the increased IS observed is indeed due to a lack of dFMR1.

Does elevated IS lead to the circadian and cognitive phenotypes? To address this, the authors expressed a dominant-negative form of the 110-kDa catalytic subunit of PI3K ($DP110^{DN}$) which reduces its activity, or the phosphatase and tensin homolog protein (PTEN) which antagonizes PI3K activity. Both of these manipulations significantly increased the rhythmicity and amplitude of circadian rhythms in d*fmr1* mutants, rescuing the circadian defects. $DP110^{DN}$ and PTEN expression also rescued the

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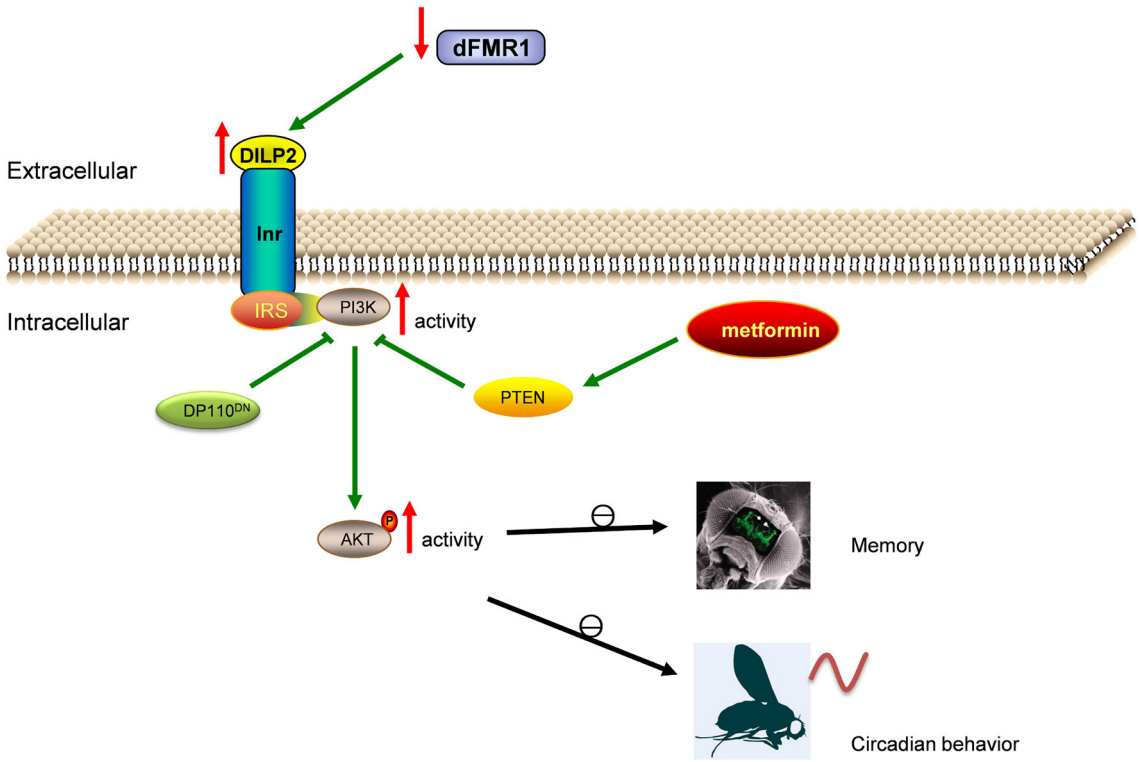

Fig. 1 Enhanced insulin signaling underlies circadian rhythm and memory defects in the *Drosophila* model of FXS. The DILP2 protein level is elevated when dFMR1 is reduced, resulting in increased activity of PI3K. Activated PI3K leads to phosphorylation and activation of AKT, ultimately contributing to the deficits in circadian rhythm and memory. Expressing $DP110^{DN}$ and PTEN suppresses PI3K activity and thus can rescue the deficits of circadian rhythm and memory. Metformin may reverse the deficits by increasing PTEN expression.

performance index of d*fmr1* mutants in courtship-based (short-term) and olfactory-based (long-term) memory tests, indicating a general improvement in memory when IS/PI3K signaling is reduced.

The authors then took one step further and fed d*fmr1* mutant flies with metformin, a drug used to treat type 2 diabetes. Metformin increases PTEN expression and AMPK activation, while decreasing TOR signaling. Although metformin failed to rescue the circadian deficits, it significantly improved performance in both short- and long-term memory tests. The authors later demonstrated that IS needs to be reduced specifically during the pupal stage to rescue the circadian phenotypes. However, flies do not ingest any food during this stage and thus cannot consume any metformin. This may explain the lack of improvement in circadian rhythms when flies are supplied with metformin in the food.

Previous studies have identified several signaling pathways that are involved in the pathological processes underlying FXS, including the metabotrophic glutamate receptor-MAPK pathway, the mTOR pathway, and the cAMP pathway [9–11]. Current treatments either focus on restoring the excitatory/inhibitory balance of neurotransmission and modifying FMR1 targets, or aim at relieving the symptoms, but none have been satisfactory [3]. Metformin, being a drug already approved by the US Food and Drug Administration, if proven to be effective in FXS patients, could be put into use rapidly.

IS is known to be regulated by the circadian clock, playing an important role in the crosstalk between the circadian clock and metabolism [12]. Conversely, IS also regulates the amplitude of the molecular clock both in flies and in mammals [13, 14]. Given that a lack of dFMR1 alters clock output, that reducing IS in d*fmr1* mutants restores locomotor rhythms suggests a role for IS downstream of the clock in the output pathway regulating behavioral rhythms [6, 7]. It would be interesting to determine whether IS functions in the circadian output pathway to control locomotor rhythms in wild-type animals as well.

IS is known to regulate synaptic plasticity and a strong association exists between dementia and insulin resistance in the central nervous system [15]. Intranasal insulin has been shown to improve memory or cognitive function in human subjects. Mice with impaired IS exhibit defective short- and long-term memory, while enhancing IS improves these functions. Consistently, IS is required for learning and short- and long-term memory in *Drosophila* as well [16, 17]. A caveat is that most previous studies

demonstrate that a reduction in IS leads to deficits in memory or cognitive function, while d*fmr1* mutants show increased IS which also appears to disrupt memory. Given that IS modulates synaptic plasticity, a delicate balance may be necessary for optimal synaptic function, whereas too much and too little IS are both detrimental, resulting in cognitive impairment.

It has been reported that for ~75% of all human disease-related genes, homologous genes exist in *Drosophila* [18]. The current study used the fly FXS model to successfully reveal a new pathway underlying the pathology of FXS, and moreover, to identify a drug that can potentially improve the symptoms (Fig. 1). This adds further evidence demonstrating the feasibility of using flies to understand the causes of mental diseases and to screen for effective drugs.

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