

Fragile phagocytes: FMRP positively regulates engulfment activity

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Defective immune system function is implicated in autism spectrum disorders, including Fragile X syndrome. In this issue, O'Connor et al. (2017. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201607093>) demonstrate that phagocytic activity of systemic immune cells is compromised in a *Drosophila melanogaster* model of Fragile X, highlighting intriguing new mechanistic connections between FMRP, innate immunity, and abnormal development.

Fragile X syndrome is the most common heritable cause of autism spectrum disorder (Estes and McAllister, 2015). Patients present with a wide range of behavioral and neurological symptoms, including intellectual disability, hypersensitivity to sensory stimuli, sleep disorders, and impaired social proficiency. Affected individuals may also display joint hypermobility, macrocephaly, and male macroorchidism. In these patients, expansion of CGG repeats in the noncoding 5' region of the *Fragile X mental retardation protein (FMR1)* gene results in transcriptional silencing and loss of the gene product FMR1 protein (FMRP), an RNA binding protein that is believed to govern mRNA localization within cells and negatively regulate translation of bound transcripts. Across species, FMRP is ubiquitously expressed but highly enriched in the brain, mirroring the fact that morphological and functional defects appear to be most prominent in, although not restricted to, the central nervous system (CNS) in Fragile X syndrome. In patients and rodent models of Fragile X, neurons display a high density of immature dendritic spines, suggesting a failure to form or stabilize mature synapses or a failure to eliminate weak connections (Pfeiffer and Huber, 2009). Similarly, in *Fmr1* mutant *Drosophila*, neurons in the central and peripheral nervous system are overelaborated and maintain excessive immature synaptic outgrowths (Tessier and Broadie, 2008). Notably, forced expression of human *FMR1* in *Fmr1* knockout flies rescues these synaptic architecture defects, as well as male sterility. Together, these findings validate *FMR1* animal mutants as powerful *in vivo* models to elucidate the dysfunctional cellular and molecular events that underlie the pathophysiology of Fragile X syndrome.

Although it is clear that loss of neuronal FMR1 cell autonomously contributes to abnormal neuronal development and plasticity, recent work provides compelling evidence that Fragile X syndrome, as well as related autism spectrum disorders, may also be coupled to defective immune system function. Autistic individuals often have an increased incidence of autoimmune disorders, and pharmacological studies targeting

immune system components have provided exciting prospects for therapeutic strategies that target immune cells' responses (Estes and McAllister, 2015). For example, the immunosuppressive tetracycline derivative minocycline relieves synaptic defects and behavioral phenotypes in *FMR1* knockout mice and reverses neuronal connectivity abnormalities in *Fmr1* mutant *Drosophila* in both the CNS and the peripheral nervous system (Estes and McAllister, 2015). Preliminary human trials also suggest that minocycline improves neurological symptoms in Fragile X syndrome patients (Paribello et al., 2010; Leigh et al., 2013). The precise therapeutic mechanisms of minocycline are still unclear. It may directly influence synaptic structural remodeling and glial immunity or indirectly alter systemic immune function in a manner that ultimately influences CNS plasticity and behavior.

In this issue, O'Connor et al. reveal that innate immunity and, in particular, engulfment activity of professional phagocytes is compromised in Fragile X model flies (Fig. 1). In adult *Drosophila*, circulating hemocytes, which are the functional equivalent of vertebrate macrophages, perform immune surveillance and tissue repair. Bacterial injection in adult flies is a well-established method to assess the coordinated immune response mounted by hemocytes and the capacity for these cells to clear invading pathogens (Gold and Brückner, 2015). O'Connor et al. (2017) demonstrated that *Fmr1* mutant animals had reduced resistance to infection after injection of *Streptococcus pneumoniae* or *Serratia marcescens*. More specifically, hemocyte depletion of *Fmr1* inhibited hemocyte engulfment of bacteria and shortened the lifespan of adults. Hemocyte numbers were unaffected and core innate immunity pathways (e.g., Toll and imd) were activated normally, suggesting that hemocytes develop, migrate, and detect infection normally in Fragile X model flies. Notably, resistance to *Listeria monocytogenes* or *Pseudomonas aeruginosa* bacterial infection was normal in *Fmr1* mutants, indicating that *Fmr1* is likely required in hemocytes to recognize or internalize particular forms of bacteria.

As in the vertebrate nervous system, the developing *Drosophila* CNS undergoes dramatic sculpting and refinement to generate proper adult circuitry (Corty and Freeman, 2013). Superfluous neurons are destroyed through programmed cell death, and many immature neurons extend excessive projections that must eventually be pruned. Local ensheathing glia and astrocyte-like glia are responsible for clearing these spent cells and unnecessary projections, and defects in glial phagocytic

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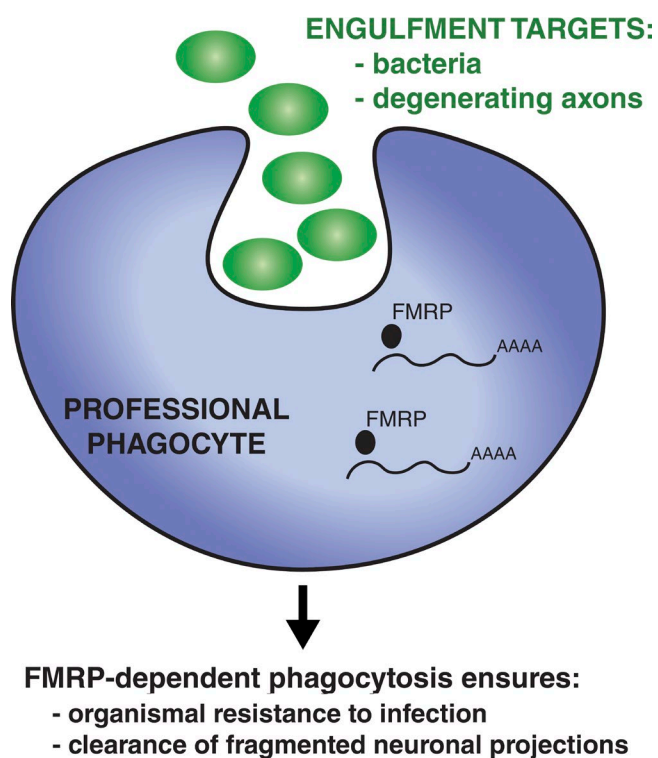


Figure 1. Schematic of FMRP-mediated phagocytic function in *Drosophila*. The RNA-binding protein FMRP functions cell autonomously in systemic adult hemocytes to promote phagocytic clearance of bacterial pathogens. FMRP may similarly promote glial clearance of degenerating neuronal projections during CNS development and after acute axon injury. Loss of FMRP, an RNA binding protein that typically inhibits translation of target transcripts, likely results in overproduction of as-yet-undefined factors that negatively regulate phagocytic function in vivo.

function result in improper neuronal wiring in adult flies (Corty and Freeman, 2013). Notably, phagocytic glial cells and hemocytes rely on some common intracellular signaling pathways to internalize and destroy engulfment targets (Corty and Freeman, 2013). Thus, O'Connor et al. (2017) assessed axonal pruning in the adult mushroom body (MB), the learning and memory center of the brain in *Fmr1* mutant flies. Like many regions in the *Drosophila* nervous system, the MB undergoes extensive remodeling during pupal metamorphosis. Larval MB axonal projections are pruned to accommodate new outgrowth and the formation of adult-specific networks in the CNS; these pruned MB axons are phagocytically cleared by local glial cells (Corty and Freeman, 2013). O'Connor et al. (2017) showed that clearance of MB neuron axons was delayed in newly eclosed *Fmr1* mutants. This intriguing observation supports previous work that revealed *Fmr1*^{-/-} MB neurons, generated through single cell MARCM clone strategies, displayed excessive branching and increased synaptic density (Tessier and Broadie, 2008). It remains to be determined how much of the MB axon pruning defects are cell autonomous and resultant from neuronal depletion of Fmr1 and how loss of Fmr1 in glial cells contributes to pruning delays. Nonetheless, the notion that glial-specific Fmr1 activity influences neuronal elaborations and circuitry and contributes to Fragile X syndrome pathology is gaining validity. For example, recent work from Hodges et al. (2016) revealed that selective deletion of *Fmr1* from mouse astrocytes resulted in increased spine density and learning defects in adult mice. Together, these studies provide exciting new evidence that

cooperative interactions between glial cells and neurons may underlie Fmr1-mediated sculpting of the CNS across species.

In the adult brain, protective glial responses, including enhanced engulfment activity, are elicited in response to acute injury or disease-induced neurodegeneration. Efficient glial clearance of dying neurons and degenerating projections attenuates inflammation and minimizes CNS damage. Recent work also implicates glial engulfment of mature synapses as an underlying factor contributing to the onset and progression of neurodegenerative disorders, including Alzheimer's disease (Hong et al., 2016). Interestingly, these phagocytic responses appear to reflect reactivation of pathways that are normally used during developmental glial pruning (Hong et al., 2016). With this in mind, O'Connor et al. (2017) asked if phagocytic function was altered in adult Fragile X syndrome model flies. They used a well-established in vivo *Drosophila* axotomy assay, which entails severing the olfactory nerves that project into the central brain, and monitored glial clearance of these degenerating olfactory axons. Indeed, they found that glial phagocytic clearance of severed axons was delayed in *Fmr1* mutant flies. This phenotype may be a result of glial-specific defects that interfere with the ability to sense injury, recognize damaged axons, or internalize fragmented projections. Alternatively, Fmr1 may be required both in adult axons to drive a proper Wallerian degeneration program and in glia to facilitate phagocytic responses, which would indicate that Fmr1 is required for cooperative neuron–glia interactions in the developing and adult CNS.

FMRP/Fmr1 is defined as an RNA-binding protein that associates with polyribosomes, typically to inhibit translation of select mRNAs. Some pressing questions raised by the work of O'Connor et al. (2017) include the following: What are the key transcripts targeted by Fmr1 in professional phagocytes? How does overproduction of these targets inhibit engulfment activity in hemocytes and in glial cells? Could boosting phagocytic activity of systemic immune cells or CNS glia reverse Fragile X syndrome symptoms? There are a number of intriguing candidate molecules that may be regulated by Fmr1 in phagocytes. In developing *Drosophila* neurons, Fmr1 genetically interacts with the GTPase Rac1 and directly inhibits translation of the actin-binding molecule Profilin to influence cytoskeletal dynamics and neuronal outgrowth (Tessier and Broadie, 2008). Because Rac1-mediated morphogenic responses drive engulfment activity of systemic phagocytes and glia, Rac1/Profilin and related cytoskeletal regulators represent interesting putative Fmr1 targets in these cell types. The *Drosophila* model of Fragile X syndrome will undoubtedly continue to provide a tractable genetic system to identify novel factors that couple Fmr1 to phagocytic function and innate glial immunity. Immune cell-specific analyses, combined with proteomics approaches across species, will also be essential to generate a comprehensive picture of FMRP/Fmr1 functions in various tissues and contexts. Overall, the work of O'Connor et al. (2017) forces us to reframe how we view FMRP's influence on the immune system, as well as in the CNS, and will open new avenues to develop effective treatments for Fragile X syndrome patients.

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