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Spotlight

TRIMing Neural Connections with Ubiquitin

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During evolution, ubiquitin ligases increased in number with increasing nervous system complexity. Recent work shows that proper brain development, cognitive ability, and social behavior in mice require the ubiquitin ligase TRIM67. The work illuminates how general regulators like ubiquitin promote specific functions such as nervous system wiring during development.

Some discoveries produce a Eureka moment. The discovery of the HOX code, for example, revealed deep and surprising evolutionary conservation of the molecular mechanisms that pattern the bodies of animals as diverse as flies and humans. Other discoveries produce years or decades of head-scratching before their significance becomes evident. Mendel's rules of inheritance come to mind. If you are grappling with perplexing results, take heart. Sometimes we have to chip away at our ignorance for decades before we see what our results mean, like archaeologists meticulously chiseling amazing fossils out of chunks of rock. The roles of ubiquitin ligases in the control of neuronal connectivity and animal behavior fall into this latter category. Piece by piece, a picture is gradually emerging, providing unexpected insights into a fundamental problem in developmental neurobiology and animal behavior.

In recent work, Boyer et al. (Boyer et al., 2018) generate knockout mice and use a suite of behavioral assays to clarify the physiological importance of a specific ubiquitin ligase, called TRIM67, which attaches ubiquitin peptides to target proteins. TRIM67 mutants show defects in the development of specific brain regions and neural functions such as spatial memory, cognitive flexibility, and social behavior. Biochemically, TRIM67 heterodimerizes with its closest paralog TRIM9 and binds to Deleted in Colorectal Cancer (DCC), a well-studied neuronal pathfinding receptor. The work raises exciting questions for future study including whether genetic variations in this locus affect human cognitive development.

To place the new work in context, let's go back to the 1980s, when neurobiologists first set out to solve the great puzzle as to how the nervous system wires itself up during development. How the 80 billion neurons of the human brain make specific connections is a problem of mind-boggling complexity. Since puzzles with fewer pieces are easier to solve, a handful of investigators sought to decipher neural development by studying organisms with simpler nervous systems. For example, John Thomas and Bob Wyman devised an elegant screen to identify mutant fruit flies bearing an exquisitely specific defect in nervous system wiring and behavior (Thomas and Wyman, 1982). They named their favorite mutant *bendless* because one axon bundle fails to turn away from the midline at the characteristic place where it normally bends. It took a decade for DNA cloning techniques to enable identification of the mutated gene. The expectation was that it would reveal the secret of neuronal wiring much as the HOX code demystified the evolution and development of segmented body plans. The specificity of the *bendless* phenotype suggested that the gene would encode a nervous-system-specific protein. More precisely, *bendless* was expected to encode a transmembrane receptor for an extracellular cue that would cause growth cones to turn instead of stopping or proceeding straight. The molecular nature of neuronal turn-by-turn instructions was about to be uncovered.

Instead, they got a head-scratcher. The *bendless* gene encoded one of the seemingly least-specific proteins imaginable, an E2 ubiquitin ligase (Muralidhar and Thomas, 1993). E2 ligases move ubiquitin from E1 proteins to E3 enzymes, which covalently attach ubiquitin peptides to substrates (Varshavsky, 2012). How could ubiquitin, which as its name suggests is everywhere, have such a specific function? Not only is ubiquitin ubiquitous, but its best-characterized function was targeting misfolded proteins to the cell's garbage disposal. This was not a Eureka moment.

As another decade passed, many more neuronal pathfinding molecules were identified. Iconic examples include Unc6/Netrin and their Unc40/Frazzled/DCC receptors, first identified in *C. elegans* (Hedgecock et al., 1990). In animals from worms and flies to mice and men, these molecules steer growth cones to the midline forming structures called commissures. The idea that simple organisms would share and reveal fundamental mechanisms has held up well.

By 2010, several ubiquitin ligases were known to function in neural development, and one particular E3, TRIM9, was firmly implicated in the Unc40/DCC pathway in worms (Hao et al., 2010). It was still not obvious, though, how ubiquitin could direct pathfinding. The nervous system is capable of such extraordinary feats, like reading and understanding this article, that it is almost easy to forget that neurons are still cells that evolved from non-neuronal cells. So, neurons typically modify and elaborate upon common molecular machinery to carry out their very special tasks. Synaptic vesicles, for example, are a specialized form of the secretory vesicles that most cells possess. Ubiquitination is another great example.

As we now know, ubiquitination is a versatile post-translational modification that exerts diverse effects on target proteins, and there are more E3 ubiquitin ligases encoded in the human genome than there are protein kinases (Deshaies and Joazeiro, 2009). During evolution, the family of E3 ligases expanded in number and diversity as animals, and their nervous systems, gained complexity. Expression of many E3 ligases is enriched in the nervous

system. Therefore, ubiquitin ligases are diverse and specific enough to participate in precise functions like axon pathfinding.

A key mechanistic insight into the role of ubiquitin in axon guidance came from the Gupton lab in 2015 (Menon et al., 2015). This work demonstrated that TRIM9 localizes to the tips of growth cone filopodia where it ubiquitinates VASP, which promotes F-actin polymerization. Ubiquitinated VASP delocalizes from filopodia, thus destabilizing them. Together with a deubiquitinating enzyme, TRIM9 forms a gradient of ubiquitinated VASP that determines which filopodia will be stable and, thus, which direction growth cones will turn to steer axons in response to Netrin/DCC. TRIM9-dependent ubiquitination of DCC also restricts axon outgrowth by blocking intracellular signaling and exocytosis of new membrane (Plooster et al., 2017).

Defining the physiological functions of the vast number of ubiquitin ligases is a major goal for the field (Deshaies and Joazeiro, 2009). In filling this gap for TRIM67, Boyer et al. (2018) uncovered intriguing phenotypes. The authors chose TRIM67 because it is closely related to TRIM9. The single worm homolog of the TRIM9/TRIM67 paralog pair functions downstream of Unc40 (worm DCC), and fly TRIM9/TRIM67 functions with Frazzled (fly DCC). However, mouse TRIM9 knockouts (Winkle et al., 2016) do not phenocopy DCC mutants. Boyer et al. reasoned that TRIM67 might be part of the answer. They were right. They made TRIM67 knockout mice and found that, while viable, they exhibit specific defects in nervous system development. TRIM67 binds DCC and can heterodimerize with TRIM9. However, TRIM67 is also expressed earlier in development than TRIM9 and so may also have TRIM9-independent functions. For example, TRIM67 may homodimerize or heterodimerize with more distant relatives.

TRIM67 phenotypes overlap with DCC mutants. For example, the corpus callosum and hippocampal commissure are reduced in size in TRIM67 mutants and eliminated in Netrin or DCC mutants. Perhaps a TRIM9/TRIM67 double mutant will phenocopy DCC even more completely. The authors go on to document specific cognitive and behavioral deficits in the TRIM67 mutant animals, likely consequences of the developmental anomalies.

Eureka moments are exciting, but stories like this one are also deeply gratifying. They give us hope that today's baffling result might just be the first lonely piece in an amazing puzzle. That a ubiquitin ligase affects development of specific axon tracts and selective mammalian behaviors, as shown by Boyer et al. (2018), finally fulfills the vision of the developmental neurobiology pioneers.

This work, like all good studies, raises fascinating questions. Do alterations in the TRIM67 gene affect human brain development and cognitive function? Could even subtle allelic variation in E3 ligases generate diversity in neural connectivity and intellectual ability? Does TRIM67 function like TRIM9 at filopodial tips? Or are there as yet unidentified substrates and mechanisms by which TRIM E3 ligases affect neural development? While many pieces have fallen into place over the past 40 years, this puzzle is still far from complete.

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