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SPOTLIGHT

BRAG about (s)lots

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Mutations in IQSEC2/BRAG1 cause intellectual dysfunction by impairing ARF-GEF activity and long-term depression. In this issue, Bai et al. (https://doi.org/10.1083/jcb.202307117) discover how constitutive ARF-GEF activity is regulated by a closed conformation which opens in the presence of Ca^{2+}. Two known pathogenic mutations cause "leaky" autoinhibition with reduced synaptic dynamic range and impaired cognitive performance.

IQSEC2, the X-linked gene encoding BRAG1, is being implicated in a rapidly growing number of identified neurodevelopmental disorders causing intellectual disability, encephalopathy, autistic symptoms, and epilepsy (1). These diseases are often caused by synaptic dysfunction such as dysregulation of trafficking of AMPA-type glutamate receptors and mediate basal postsynaptic responses at glutamatergic synapses, the prevalent types of synapses in the forebrain. BRAG1 has a unique bidirectional role in trafficking of AMPA receptors (2). BRAG1 facilitates AMPA receptor removal and long-term depression (LTD) through activation of ARF GTPases by the guanine nucleotide exchange factor (GEF) function of its Sec7 domain (3, 4). On the other hand, BRAG1 can also augment postsynaptic AMPA receptor content, independent of synaptic activity, via a C-terminal PDZ binding domain (2).

To prevent receptor degradation and preserve molecular memory, the constitutively recycling AMPA receptor containing GluA2 subunits replaces mostly GluA1-containing AMPA receptors around 18 h after long-term potentiation (LTP) induction (5). This finding supported the hypothesis of a "slot" protein, which is proposed to retain the correct number of AMPA receptors by establishing a designated space at the synapse (6). Considering that BRAG1 removes GluA1 (4) through GEF activity and increases GluA2 (2) expression through PDZ scaffolding interactions (7), the question arises whether BRAG1 acts as such a slot protein, thereby mediating AMPA receptor subtype exchange. Having a single protein remove GluA1 and arrange a slot for GluA2 would constitute a perfect design by ensuring tight regulation of receptor expression. This speculative role for BRAG1 could constitute a molecular mechanism for memory consolidation, which is driven by LTP of various glutamatergic synapses.

Most of the clinical attention on IQSEC2/BRAG1 has focused on its Arf-GEF activity. Mutations of the Sec7 domain and calmodulin (CaM) binding IQ (isoleucine-glutamate)-like motif both cause reduced GEF activity, impaired LTD, and intellectual disability (2, 4, 8). BRAG1 knockout mice display autistic-like deficits also consistent with impaired LTD (9). Yet, until this issue's report by Bai and colleagues (10), it had not been determined how BRAG1's constitutive GEF activity is regulated, or precisely how these clinical mutations lead to impaired GEF activity and subsequent synaptic and cognitive deficits.

Because alterations in the IQ and Sec7 domains have overlapping phenotypes, Bai et al. (10) first sought to understand the nature of their interactions. They found that in the absence of Ca^{2+}, the Apo-CaM/IQ complex tightly binds the Sec7-PH tandem resulting in a closed conformation, which silences Sec7 catalytic activity. They also determined the crystal structure of the IQSECs, which revealed a three-layer complex with Sec7-PH on bottom, Apo-CaM on top, and an L-shaped IQ motif in the middle comprised of two connected α helices. Both helices are necessary for IQ/Sec7 binding and may explain why the IQSEC family of IQ motifs are three times larger than canonical IQ motifs. The closed conformation opens when Ca^{2+} binds CaM (Ca^{2+}-CaM), which reduces affinity for Sec7 by 100-fold. With Sec7 exposed, the constitutively active GEF is unleashed. Indeed, fluorescence-based assays demonstrated that Ca^{2+}-CaM allowed for an approximately fivefold increase in GEF activity compared to Apo-CaM, and yet is only half that of isolated Sec7-PH, indicating there is still some structural interference by an intact Ca^{2+}-CaM/IQ complex. The crystal structure of CaM predicts that its C-lobe binds IQ and its N-lobe projects away due to weakened binding caused by the Gln/Ser substitution for Arg. This orientation allows for direct contact with Sec7. In all, there were five reported binding sites of the Apo-CaM/IQ/Sec7-PH ternary complex, with most clinical mutations predicted to weaken or abolish these interactions.

Having elucidated the Ca^{2+}-regulated autoinhibition mechanism of BRAG1, Bai and colleagues were in position to interpret the molecular basis of reported pathogenic human mutations. Their structural model predicted that replacing Q801 with Proline

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in the Sec7 domain should destabilize the α4 helix, alter its conformation, and impair GEF activity and LTD. Consistent with prior observation (8), GEF activity was decreased, but the dynamic Ca^{2+}-mediated regulation was preserved.

Bai et al. (10) then examined two known pathogenic IQ mutations, R359C and A350V/D. These mutations weakened the interaction between IQ and Apo-CaM but had less impact on the Ca^{2+}-CaM binding. The result was higher basal GEF activity (relative to Q801P) due to weaker autoinhibition, but with less responsiveness to Ca^{2+} and resultant GEF activation. In other words, R359C caused a "leaky" and inefficient enzyme with decreased dynamic range. It is interesting to note that while the A350D mutation had higher basal GEF activity, dynamic range enabled by Ca^{2+}-mediated release of autoinhibition was abolished. Dynamic range, which dictates synaptic plasticity, may be the critical element for normal brain function as suggested by the more severe disease phenotype associated with the A350D mutation.

To better understand how these molecular changes correspond to synaptic and cognitive function, Bai and colleagues generated mice with the R359C and Q801P mutations. Both mice were normal in body weight, fertility, brain morphology, neuropsychiatric-oriented tests, and indices of GABAergic function and glutamatergic synapse numbers. Both had impaired LTD with corresponding LTD-dependent relearning deficits, as would be expected from reduced GEF activity. Loss of LTD in other mutant mice had been linked to impaired reversal learning the Morris Water Maze (MWM) during which the escape platform is moved to the side of the pool opposite to the original platform location (11, 12). Consistently, Bai et al. (10) found that this reversal learning was strongly impaired in both of their mutant mouse strains. At the same time, the R359C mouse mutant also showed strongly impaired learning of the initial platform location, an impairment that was minor, if not absent, in the newly created Q801P as well as the mutant earlier mouse strains.

Particularly striking were how the differences between these mice aligned enzymatic, synaptic, and behavioral phenotypes. The Q801P mice, which retained a dynamic range for Ca^{2+} responsiveness, correspondingly had intact LTP, spatial memory, and novelty seeking. However, they suffered from lower baseline GEF activity, which equated to decreased basal synaptic transmission, and markedly less exploratory activity and volition on multiple behavioral tests. It may be worth noting that Brown et al. (2) showed increased synaptic transmission, though this was after acute transfection, whereas the Q801P mice would have had chronic LTD impairment and subsequent overexcitation, which may have led to homeostatic reduction of basal AMPA receptor expression.

By contrast, the R359C mice, which had closer-to-normal-baseline enzymatic GEF activity, displayed concordant characteristics of normal basal synaptic transmission and normal amount of time exploring a novel object versus a familiar object. However, they had a blunted range of GEF activity in response to Ca^{2+}, with analogous blunting of LTP and spatial learning in the MWM original platform location. In fact, the R359C mice exhibited several classic autistic behaviors including overgrooming and a striking lack of social interaction, i.e., a lack of preference for unfamiliar mice versus a familiar object.

In summary, Bai et al. (10) provide a huge step forward in our understanding of IQSEC2/BRAG1 function and pathology, which opens the door to generating personalized precision treatment strategies.

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