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In a Pickle: Is Cornichon Just Relish or Part of the Main Dish?

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The recent discovery that vertebrate homologs of *Drosophila* cornichon associate with AMPA receptors led to the unexpected notion that cornichons play a role in synaptic transmission. In this issue of *Neuron*, Kato et al. find that cornichons modulate the gating of TARP-associated AMPA receptors by preventing their resensitization to glutamate.

Excitatory synaptic transmission in the brains of most animals is mediated primarily by the neurotransmitter glutamate—a ubiquitous amino acid with diverse actions on neuronal excitability. Different classes of cation-permeable (ionotropic) transmembrane receptor proteins mediate rapid excitatory synaptic signaling by glutamate (Dingledine et al., 1999). One class of these ionotropic receptors (AMPARs) are found at most brain synapses, and different patterns of synaptic transmission can lead to stable changes in AMPAR properties and numbers. These experience-dependent changes modify the efficacy of synaptic transmission in cellular models of learning and memory such as long-term potentiation (LTP) and long-term depression (LTD) (Kessels and Malinow, 2009). AMPARs were initially believed to be stand-alone receptors; however, genetic and biochemical studies have now firmly established that the localization and function of AMPARs, and perhaps of all ionotropic glutamate receptors, depend on auxiliary proteins.

Studies of stargazer mutant mice led to the discovery of stargazin, the first identified AMPAR auxiliary protein and the founding member of the *t*ransmembrane *A*MPA receptor *r*egulatory *p*rotein (TARP) family, which also includes γ-3, γ-4, γ-5, γ-7, and γ-8 (Milstein and Nicoll, 2008; Kato et al., 2010b). TARPs are physically associated with AMPARs; contribute to their trafficking, synaptic localization, and channel conductance; and, importantly, slow the rates of receptor deactivation and desensitization. Independent studies in *C. elegans* identified two genes that encode the TARPs STG-1 and STG-2, which appear to make up the complete TARP family in *C. elegans.* AMPAR-mediated currents cannot be detected in the *stg-1; stg-2* double mutant (Wang et al., 2008), thus demonstrating the central importance of TARPs for AMPAR function. TARP function is also evolutionarily conserved as demonstrated in reconstitution experiments with *C. elegans* TARPs and vertebrate AMPARs. Thus, studies in vastly different organisms highlight the importance of TARPs for AMPAR function and support the hypothesis that the majority of AMPARs are associated with TARPs (Milstein and Nicoll, 2008; Kato et al., 2010b). Additional evidence in support of this hypothesis was provided by cryo-EM studies of purified AMPARs (Nakagawa et al., 2005).

The discovery of TARPs helped solve the puzzle of why the kinetic and pharmacological properties of native neuronal AMPARs did not match those of AMPARs expressed in heterologous cells. At first glance, TARPs appeared sufficient for AMPAR function, and thus there was no apparent need to invoke the possibility of additional auxiliary proteins. However, our understanding of AMPAR biology is far from complete largely because of the limited tools and paradigms available to evaluate synaptic receptors. Perhaps there are additional auxiliary proteins. A relatively unbiased and straightforward approach to test this possibility is to simply ask this question: what proteins are associated with AMPARs? Schwenk et al. (2009) did just that by affinity purifying AMPARs from rat brain followed by a proteomic approach to identify interacting proteins. As expected, they found TARPs. However, they also found that AMPARs associated with CNIH-2 and CNIH-3, which are vertebrate homologs of *Drosophila* cornichon (French for "pickled gherkin"). This small transmembrane protein is highly conserved and known family members have chaperone roles in the export of select secretory and transmembrane cargo from the endoplasmic reticulum (ER) (Jackson and Nicoll, 2009).

In reconstitution studies, CNIHs increased AMPAR surface expression and had dramatic effects on AMPAR kinetics. In fact, CNIHs' slowing of AMPAR deactivation and desensitization was greater than that observed for comparable reconstitution experiments using TARPs. Immuno-EM studies identified CNIHs in dendritic shafts, in spines, and in the postsynaptic density (PSD), suggesting that they could function as bona fide AMPAR auxiliary proteins rather than simply as chaperones. Approximately 70% of AMPARs were associated with CNIHs, but not with TARPs; similarly, the 30% of receptors associated with TARPs were not associated with CNIHs. At first blush, mutually exclusive auxiliary proteins that associate with AMPARs appeared incompatible with previous genetic and biochemical studies that support the hypothesis that the majority of functional AMPARs are associated with TARPs. Regardless, it is difficult to discount the dramatic effects on channel kinetics that were observed when CNIHs were coexpressed with AMPARs in heterologous cells. Either this was a nonspecific effect, which seems unlikely, or CNIHs have a fundamental role in some aspect of AMPAR biology.

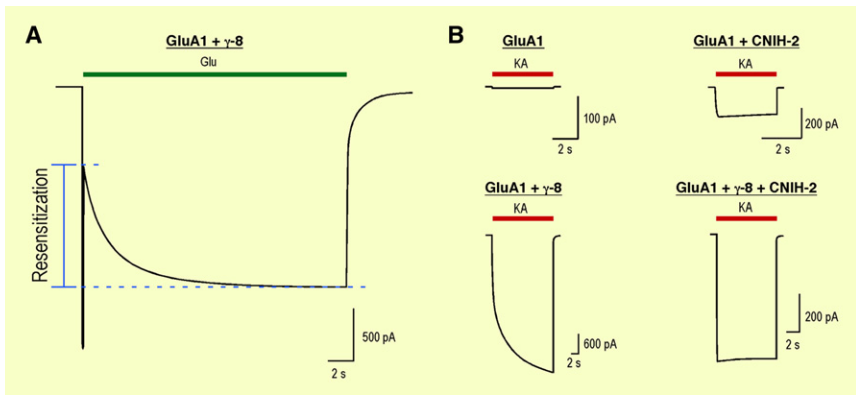

Figure 1. It Takes Two to Control AMPA Receptor Gating in the Hippocampus
Kato et al. (2010a) show that AMPARs coexpressed with the TARP γ-8 in HEK cells exhibit resensitization in the continued presence of glutamate (A) or kainate (B). However, most hippocampal AMPARs are associated with γ-8, yet do not exhibit resensitization. The missing piece appears to be the cornichon CNIH-2. The gating and pharmacology of AMPARs coexpressed with both γ-8 and CNIH-2 in HEK cells are similar to that of native hippocampal receptors and resensitization is abolished (B). Figure adapted from Kato et al. (2010a).

In this issue of Neuron, Kato et al. (2010a) approached the study of AMPAR function from a different angle. They first asked whether reconstituted AMPARs in HEK cells behave like native hippocampal receptors. Whereas most biophysical studies of AMPARs measure the rapid kinetics of receptor deactivation and inactivation (on the order of ms), Kato et al. (2010a) measured currents during prolonged applications of agonist (many seconds) and discovered a new phenomenon that they called resensitization (Figure 1). What they observed after agonist application was an initial rapid but incomplete desensitization of current, followed by a slow increase in current amplitude, i.e., a reversal of desensitization in the continued presence of glutamate or kainate. Resensitization was only observed when AMPARs were coexpressed in HEK cells with a subset of known TARPs (γ-4, γ-7, or γ-8) and was not observed when AMPARs alone were expressed in HEK cells or when they were coexpressed with γ-2, γ-3, or γ-5. In contrast, native hippocampal AMPARs do not resensitize, yet most AMPARs in hippocampal neurons are associated with γ-8. These results suggested that protein(s) in addition to γ-8 contribute to AMPAR function in vivo by preventing TARP-mediated resensitization. The authors tested the hypothesis that CNIH proteins might constitute this missing component. They found that the properties of AMPARs coexpressed in HEK cells with either γ-8 or CNIH-2 differed from each other and from those of native hippocampal receptors. However, AMPARs coexpressed with both γ-8 and CNIH-2 did not resensitize and also exhibited the pharmacological properties of native hippocampal receptors. Thus, Kato et al. (2010a) provide evidence for an AMPAR complex containing both TARPs and CNIHs and showed that these auxiliary proteins have distinct roles in modulating receptor function.

Although TARPs are enriched at the PSD (Tomita et al., 2003), whether CNIHs are also enriched had not been addressed. Using a biochemical approach, Kato et al. (2010a) found that GluA1, γ-8, and CNIH-2 were all similarly enriched in PSD subcellular fractions from brain extracts. These findings nicely complemented the earlier immuno-EM studies of Schwenk et al. (2009) and provided further support for a tripartite complex in hippocampal neurons consisting of GluA1, γ-8, and CNIH-2. In addition, CNIH-2 was detected at the cell surface by using biotinylation reagents; association of CNIH-2 and TARPs was demonstrated by coimmunoprecipitation; and immunofluorescence experiments revealed that CNIH-2 colocalized with both γ-8 and GluA1 along dendritic spines (although it was also found elsewhere). Finally, cyclothiazide modulation of AMPARs in hippocampal neurons differs from that of AMPARs coexpressed with TARPs in HEK cells. However, when GluA1, γ-8, and CNIH-2 were coexpressed in HEK cells, the efficacy of cyclothiazide approximated that of native hippocampal AMPARs.

The study by Kato et al. (2010a) revealed the new phenomenon of TARP-mediated AMPAR resensitization. By exploring the mechanism of γ-8 dependent resensitization they revealed the effect of CNIH-2 on the properties of AMPARs, thus providing further evidence for an additional level of complexity in the regulation of AMPAR function. However, this phenomenon was not observed with all TARPs, leaving open the question of whether all synaptic AMPARs are associated with CNIHs.

A recent study by Shi et al. (2010) addressed the relative contributions of CNIHs and TARPs to the trafficking and function of synaptic AMPARs. They first measured the properties of AMPARs coexpressed in HEK cells with both CNIH-2 and γ-8 and found slow kinetics, consistent with binding to CNIH-2, and an increased response to kainate, consistent with binding to γ-8. They obtained similar results when CNIH-2 was coexpressed with a TARP-AMPAR fusion construct. Together, these results support the notion that CNIHs and TARPs modulate AMPARs by interacting with distinct binding sites. However, Shi et al. (2010) found that overexpressing CNIH-2 in neurons had only a minor effect on extrasynaptic AMPARs and no evidence for a significant contribution to synaptic AMPAR function. On the contrary, the properties of synaptic AMPARs were most consistent with their exclusive association with TARPs. In support of their electrophysiological data, they found that CNIH-2 was barely detectable at the cell surface and that the majority of CNIH-2 expressed in cultured hippocampal neurons appeared associated with intracellular organelles (colocalization with the *cis*-Golgi marker GM130). This begs the question: why do CNIHs associate with surface AMPARs in HEK cells but hardly at all in neurons? One possibility is that essential cell biological processes differ between the two cell types such that neurons exclude CNIH from the plasma membrane. However, this contradicts the finding by Kato et al. (2010a) that CNIH-2 contributes to synaptic AMPAR

function in transfected neurons. Discrepancies between these two studies might reflect subtle methodological differences in the overexpression studies.

Collectively, the data on CNIHs put us in a bit of a pickle. Kato et al. (2010a) find evidence for a hippocampal tripartite receptor complex containing AMPARs, CNIHs, and TARPs. On the other hand, Schwenk et al. (2009) argue that AMPARs associate with either TARPs or CNIHs in a mutually exclusive manner. Kato et al. (2010a) provide evidence that CNIHs modulate the kinetic properties of AMPARs in neurons and HEK cells, whereas Shi et al. (2010) find that CNIHs only have significant effects on AMPARs expressed in HEK cells. How can these findings be reconciled? The most obvious starting point is the discovery of resensitization by Kato et al. (2010a), which occurs at a vastly slower timescale than conventional deactivation, desensitization, and EPSCs. Does CNIH-2 have a direct role in modulating resensitization, or an indirect role, perhaps by recruiting additional proteins to the signaling complex? It is curious that resensitization is observed with only a subset of TARPs. Do CNIHs also form tripartite complexes with AMPARs and the TARPs that do not facilitate resensitization? If so, do CNIHs contribute to AMPAR function in these complexes? Perhaps CNIHs have additional functions that are only apparent at longer timescales.

Another important question is whether there exists a sizeable pool of surface AMPARs that lack TARPs. This question demands further study, but one possibility is that both CNIHs and TARPs function as auxiliary proteins at synapses. In this scenario, most AMPARs are associated with TARPs, but a larger proportion of intracellular AMPARs are exclusively associated with CNIHs, perhaps when localized to the ER or Golgi. The studies of CNIHs are particularly interesting because the strength of synaptic transmission depends on the number of receptors localized to the synapse; the conductance of each receptor; and the amount of time the receptors conduct current after glutamate binding. That TARPs and CNIHs separately or together influence the trafficking and function of AMPARs has immediate implications for the modulation of synaptic transmission and may contribute to LTP and LTD (Kessels and Malinow, 2009). However, the definitive word on whether or how CNIHs contribute to synaptic AMPAR function awaits detailed analysis of cornichon mutants in mice or other organisms.

In the last decade, additional proteins that associate with AMPARs have been identified, starting with *C. elegans* SOL-1, a CUB-domain transmembrane protein that dramatically slows the rate of AMPAR desensitization and increases the rate of recovery from desensitization (Walker et al., 2006; Zheng et al., 2004). More recently, CKAMP44 was found to accelerate the rate of AMPAR desensitization (von Engelhardt et al., 2010), and SynDIG1 regulates the development of excitatory synapses (Kalashnikova et al., 2010). These are exciting times for the study of synaptic function. We have witnessed tremendous progress as the field has rapidly progressed from a channel-centric view to that of a receptor complex, with channel function modulated by different families of auxiliary proteins. An understanding of how these complexes are assembled, stabilized, and regulated seems essential for a mechanistic understanding of learning and memory.

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