neurons to the maximal allowable time (1.5 to 2 years). The goal is to determine whether the full repertoire of tau proteinopathies, including the development of neurofibrillary tangles, can emerge as the iPSC-derived human neurons age in the AD mouse brain. Another approach is to transplant neurons that are directly reprogrammed from older control individuals to determine whether neurons that retain aging-associated genetic and epigenetic signatures are even more susceptible than iPSC-derived neurons to developing neurodegenerative features (Mertens et al., 2015; Vierbuchen et al., 2010). A variation based on the same theme is to transplant neurons directly reprogrammed from fibroblasts from familial or late-onset AD patients into AD mouse brain to elucidate unknown mechanism(s) that contribute to the selective vulnerability of these patient-specific neurons.

Many genome-wide association studies have uncovered a growing list of genetic loci that increase the risk for AD. Results from these and others studies reinforce the idea that AD is a complex process fueled by a progressive dysregulation in interconnected mechanisms that eventually leads to cognitive decline (Canter et al., 2016; De Strooper and Karran, 2016). Given the multitude of molecular pathways that can contribute to AD pathogenesis, one ultimate challenge is to determine whether this humanized AD mouse model can serve as a vehicle to identify therapeutic interventions that are tailored based on individual needs. To accomplish this, the humanized AD mouse model will need to be significantly improved to include a diverse cellular repertoire from the human grafts, including different neuronal subtypes, astrocytes, microglia, oligodendroglia, endothelial cells, and other vascular cell types. Our ability to incorporate these cell types will hopefully help develop a fully humanized model that provides insights to disease mechanisms and therapeutic targets.

REFERENCES

Canter, R.G., Penney, J., and Tsai, L.H. (2016). Nature *539*, 187–196.

Choi, S.H., Kim, Y.H., Hebisch, M., Sliwinski, C., Lee, S., D'Avanzo, C., Chen, H., Hooli, B., Asselin, C., Muffat, J., et al. (2014). Nature *515*, 274–278.

De Strooper, B., and Karran, E. (2016). Cell *164*, 603–615.

Espuny-Camacho, I., Michelsen, K.A., Gall, D., Linaro, D., Hasche, A., Bonnefont, J., Bali, C., Orduz, D., Bilheu, A., Herpoel, A., et al. (2013). Neuron *77*, 440–456.

Espuny-Camacho, I., Arranz, A.M., Fiers, M., Snellinx, A., Ando, K., Munck, S., Bonnefont, J., Lambot, L., Corthout, N., Omodho, L., et al. (2017). Neuron *93*, this issue, 1066–1081.

Mertens, J., Paquola, A.C., Ku, M., Hatch, E., Böhnke, L., Ladjevardi, S., McGrath, S., Campbell, B., Lee, H., Herdy, J.R., et al. (2015). Cell Stem Cell *17*, 705–718.

Palop, J.J., and Mucke, L. (2010). Nat. Neurosci. *13*, 812–818.

Shi, Y., Kirwan, P., Smith, J., MacLean, G., Orkin, S.H., and Livesey, F.J. (2012). Sci. Transl. Med. *4*, 124ra29.

Vierbuchen, T., Ostermeier, A., Pang, Z.P., Kokubu, Y., Südhof, T.C., and Wernig, M. (2010). Nature *463*, 1035–1041.

Zhang, B., Gaiteri, C., Bodea, L.G., Wang, Z., McElwee, J., Podtelezhnikov, A.A., Zhang, C., Xie, T., Tran, L., Dobrin, R., et al. (2013). Cell *153*, 707–720.

TARPs and AMPA Receptors: Function Follows Form

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In this issue of *Neuron*, Ben-Yaacov et al. (2017) dissect the interaction between AMPA receptors and auxiliary (TARP) subunits, revealing essential roles for the receptor transmembrane and cytoplasmic domains, as well as for the TARP extracellular EX2 loop.

Depolarizing current through AMPA receptors underlies fast excitatory transmission at glutamatergic synapses throughout the vertebrate central nervous system. Assisting AMPA receptors in this important task are an array of co-assembled auxiliary proteins (Jackson and Nicoll, 2011), including members of the transmembrane AMPA receptor regulatory protein (TARP) family (Tomita, 2010). The best-studied TARP, named stargazin or γ2 TARP, was originally identified in a mutant mouse line. Stargazer mice exhibit ataxia with characteristic head elevation and frequent spike-wave discharges typical of absence seizures that produce brief loss of consciousness in humans. Recordings from cerebellar granule cells in these mice revealed a dramatic reduction in synaptic and AMPA-evoked currents, suggesting that γ2 acts to chaperone AMPA receptor biosynthesis and/or promote forward trafficking to the surface membrane. Subsequent work led to identification of additional TARP family members as well as demonstration that co-assembly with TARPs induces a diverse array of changes in receptor kinetics and pharmacology (reviewed by Jackson and Nicoll, 2011; Tomita, 2010). Recent structural (Twomey et al., 2016; Zhao et al., 2016) and functional (Dawe et al., 2016; Ben-Yaacov et al., 2017)

studies have helped to clarify the basis for these diverse effects.

TARPS are relatively compact proteins related to the claudin family of membrane tight junction proteins. Each TARP monomer has four transmembrane (TM1 to TM4) helices with the N and C termini facing the cytoplasm. In contrast, ionotropic glutamate receptors (iGluRs) are large tetramers with each subunit contributing to four modular domains that are connected by short linkers. The extracellular amino terminal and ligand binding domains (ATD and LBD) exhibit 2-fold dimer-of-dimer symmetry, with nonequivalent A/C and B/D conformations. The 4-fold symmetric transmembrane domain (TMD) includes a pore-loop flanked by two transmembrane helices (M1-M2-M3) and an additional membrane-spanning helix (M4) that associates with M1 and M3 of the adjacent subunit. Finally, the carboxyterminal domain (CTD) contains sites for post-translational modification and binding to cytoplasmic proteins. To date, structure of the CTD has not been resolved by cryo-EM or X-ray crystallography for any of the iGluR subunits. TARPs exhibit high selectivity for AMPA receptors, with essentially no effect on other iGluRs, including the closely related kainate subtype and more distantly related NMDA receptors.

Co-assembly with TARPs increases the affinity of AMPA receptors for glutamate as well as the efficacy of partial agonists, such as kainate. In addition, the rates of desensitization in the continued presence of agonist and of deactivation upon agonist removal are slowed by TARPs. Finally, TARP co-assembly substantially reduces voltage-dependent blockade by cytoplasmic polyamines in AMPA receptors that lack Q to R editing in their pore-loops. Detailed analysis of these changes has allowed for functional subdivisions among the TARP family members. For example, type-1a TARPS (γ2 and γ3) slow deactivation rates to a lesser extent than type-1b TARPS (γ4 and γ8) (Tomita, 2010). Understanding the mechanistic basis for TARP operation has important clinical implications. Increasing evidence links aberrant TARP expression with a range of maladies including epilepsy and schizophrenia (Jackson and Nicoll, 2011). Because most of the TARP-induced changes in receptor operation serve to increase the influx of depolarizing current, drugs capable of dialing back the TARP-induced enhancement of excitatory synaptic function may be therapeutic for a variety of pathologies that involve hyperexcitability (Kato et al., 2016).

Early studies of TARP action implicated their cytoplasmic region in promotion of AMPA receptor trafficking. A motif for binding to PDZ-domain proteins at the TARP C terminus is particularly important for synaptic localization (Tomita, 2010). In addition, the EX1 extracellular loop between TARP TM1 and TM2 was shown to be important for interactions with the AMPA receptor ligand-binding domain. Within the past year, two cryo-EM studies (Twomey et al., 2016; Zhao et al., 2016) have elucidated the structure of AMPA receptors complexed with 1, 2, or 4 γ2 TARP monomers, revealing close association between γ2 TARP and the receptor transmembrane domain as well as potential electrostatic interactions between EX1 of γ2 TARP and the AMPA receptor ligand binding domain (Dawe et al., 2016).

In this issue of *Neuron*, the Stern-Bach lab uses detailed functional analysis to infer additional information about the structural requirements for AMPA receptor modulation (Ben-Yaacov et al., 2017) that complements and extends beyond what can be gleaned from the static structural views. Taking advantage of the fact that closely related kainate receptors are insensitive to TARP modulation, Ben-Yaacov et al. (2017) analyzed modulation by γ2 TARP for a series of reciprocal chimeric constructs that combined domains from AMPA receptor subunits and the GluK2 kainate receptor subunit. Their analysis focused on the ability of γ2 TARP co-expression to reduce desensitization by glutamate, enhance efficacy of kainate, reduce rectification, and increase forward trafficking to the surface membrane. The results suggest that interactions involving the receptor extracellular ligand binding domain as well as the transmembrane domain and the cytoplasmic C-terminal domain all contribute to TARP specificity for AMPA versus kainate receptor subunits, with the TMD and CTD playing particularly important roles.

Chimeric receptors with extracellular domains derived from AMPA receptors linked to the kainate receptor TMD and CTD were unaffected by TARP co-expression. Swapping in the M1-M3 pore and CTD from AMPA receptors was required for γ2-dependent regulation of desensitization to glutamate, whereas adding the pore alone was sufficient for forward trafficking and adding only the M4 helix from AMPA receptors enabled γ2-dependent regulation of kainate efficacy and current rectification in these chimeric constructs.

Surprisingly, chimeric receptors with extracellular domains derived from kainate receptors were also sensitive to TARP modulation, provided that both the M1-M3 pore and the CTD from AMPA receptors were included. In another recent study (Dawe et al., 2016), two positively charged lysine residues unique to the AMPA receptor LBD sequence were shown to be required for slowing of desensitization by TARP, but not for other modifications to AMPA receptor function. Importantly, full-length kainate receptors with lysine substitutions at the homologous positions were not susceptible to TARP modulation (Dawe et al., 2016). Taken together, these results suggest that the extracellular portions of kainate receptors can make productive contacts with TARPs, but only in the context of the AMPA receptor M1-M3 pore and CTD. Indeed, combining the full AMPA receptor TMD and CTD with the kainate receptor ATD and LBD yields channels that are constitutively active in the presence of γ2 TARP (Ben-Yaacov et al., 2017). Exposure to agonist desensitizes these chimeric channels and reduces the steady-state current below the constitutive level. Thus, TARP interaction with the AMPA receptor TMD and CTD appears to promote channel opening that is normally held in check by the extracellular domains of full-length wild-type subunits.

To shed further light on how the AMPA receptor M1-M3 pore, M4 helix, and CTD contribute to TARP modulation, Ben-Yaacov et al. (2017) co-expressed pairwise combinations of chimeric subunits that contained different subsets of these three structural elements. For example, the homomeric M1-M3 pore chimera and the homomeric M4-CTD chimera are unaffected by γ2 TARP when expressed separately. Heteromeric channels formed by co-expression of these two different chimeras display a

TARP-dependent increase in constitutive current and modulation of agonist-evoked currents typical of homomeric channels that included all three elements (M1-M3, M4, and CTD). These results, together with additional pairwise complementation experiments, reveal that TARPs interact with M1-M3 and M4 from adjacent subunits and that full complementation requires M4 and CTD both reside within the same subunit. Moreover, because heteromeric complementation will typically only reconstitute one or two sites for productive TARP interaction (Ben-Yaacov et al., 2017), these experiments confirm earlier evidence that modulation does not require the concerted action of four TARP monomers but may be achieved by action of a single TARP (Twomey et al., 2016; Zhao et al., 2016).

In addition to receptor subunit chimeras, Ben-Yaacov et al. (2017) also used chimeric constructs to examine the structural basis for more subtle differences between TARP family members. The type 1a γ2 and γ3 TARPs produced somewhat larger constitutive currents than type 1b γ4 or γ8 TARPs. Analysis of chimeric TARPs that combined segments from γ2 and γ8 revealed that the third and fourth transmembrane helices and the EX2 extracellular loop connecting them was necessary and sufficient for specifying the amplitude of constitutive current. In the cryo-EM structures, TARP TM3 and TM4 make the closest association with the receptor TMD (Twomey et al., 2016; Zhao et al., 2016), suggesting that the short EX2 loop between these TMs is ideally positioned for additional contacts with extracellular receptor domains.

Following close on the heels of the cryo-EM structures (Twomey et al., 2016; Zhao et al., 2016), these functional studies extend our understanding of how TARP modulation works (Ben-Yaacov et al., 2017) but leave many questions open for further research. In particular, evidence concerning the requirement for cytoplasmic portions both of TARPs (Jackson and Nicoll, 2011) and of AMPA receptor subunits (Ben-Yaacov et al., 2017) should spur further effort to evaluate the structure of CTD domains, alone and in association with TARPS, as well as structural rearrangements of the CTD coincident with gating (Zachariassen et al., 2016) and modulation. In addition, symmetry considerations (Twomey et al., 2016; Zhao et al., 2016) raise the possibility TARPs may interact similarly with each subunit in the 4-fold symmetric TMD (and CTD?) but exhibit non-equivalent interactions with the 2-fold symmetric extracellular domains.

TARPs regulate conformational changes in the extracellular domains including LBD closure (Jackson and Nicoll, 2011; Tomita, 2010), decoupling of ATD dimers (Shaikh et al., 2016) and other rearrangements associated with gating and desensitization. TARP contacts with subunits in the B and D configuration appear best positioned to control LBD closure (Twomey et al., 2016; Zhao et al., 2016). Future work may reveal a distinct role for TARP association with subunits in the A and C conformation, which might reconcile evidence for direct TARP contact with subregions within the ATD (Cais et al., 2014).

Resolving each of these interactions helps to clarify how TARPs may act to regulate AMPA receptor function within the complex environment at excitatory synapses (Jackson and Nicoll, 2011). Most importantly, clearer understanding of the molecular basis for receptor modulation should aid in the rational design of new therapeutic agents capable of selectively affecting interactions between specific TARP-AMPAR subunit combinations (Kato et al., 2016), allowing more selective management of pathologic excitability.

REFERENCES

Ben-Yaacov, A., Gillor, M., Haham, T., Parsai, A., Qneibi, M., and Stern-Bach, Y. (2017). Neuron *93*, this issue, 1126–1137.

Cais, O., Herguedas, B., Krol, K., Cull-Candy, S.G., Farrant, M., and Greger, I.H. (2014). Cell Rep. *9*, 728–740.

Dawe, G.B., Musgaard, M., Aurousseau, M.R., Nayeem, N., Green, T., Biggin, P.C., and Bowie, D. (2016). Neuron *89*, 1264–1276.

Jackson, A.C., and Nicoll, R.A. (2011). Neuron *70*, 178–199.

Kato, A.S., Burris, K.D., Gardinier, K.M., Gernert, D.L., Porter, W.J., Reel, J., Ding, C., Tu, Y., Schober, D.A., Lee, M.R., et al. (2016). Nat. Med. *22*, 1496–1501.

Shaikh, S.A., Dolino, D.M., Lee, G., Chatterjee, S., MacLean, D.M., Flatebo, C., Landes, C.F., and Jayaraman, V. (2016). Cell Rep. *17*, 328–335.

Tomita, S. (2010). Physiology (Bethesda) *25*, 41–49.

Twomey, E.C., Yelshanskaya, M.V., Grassucci, R.A., Frank, J., and Sobolevsky, A.I. (2016). Science *353*, 83–86.

Zachariassen, L.G., Katchan, L., Jensen, A.G., Pickering, D.S., Plested, A.J., and Kristensen, A.S. (2016). Proc. Natl. Acad. Sci. USA *113*, E3950–E3959.

Zhao, Y., Chen, S., Yoshioka, C., Baconguis, I., and Gouaux, E. (2016). Nature *536*, 108–111.