Mini-Review

Mouse Genetic Approaches to Investigating Calcium/Calmodulin-Dependent Protein Kinase II Function in Plasticity and Cognition

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The knock-out of α-calcium/calmodulin-dependent protein kinase II (CaMKII) was the kickoff for a new subfield in neuroscience, in which mouse mutants are used as a tool to gain insight into the molecular basis of cognition and brain plasticity. In our review, we give an overview of the αCaMKII mutants that have since been developed, and we summarize the key findings that these studies have provided on the function of αCaMKII in hippocampal plasticity, cortical plasticity, and learning and memory. Furthermore, we discuss recent results that misregulation of αCaMKII function may cause the neurological symptoms in Angelman's syndrome (AS).

αCaMKII mutants

To investigate the physiological role of αCaMKII *in vivo*, several mutants have now been generated (Table 1). One group of mutants was designed to manipulate the level of (active) αCaMKII. For instance, gene targeting was used to manipulate the endogenous αCaMKII gene to obtain null mutants that do not express αCaMKII protein, as well as heterozygous null mutants that express ~50% of the normal level of αCaMKII (Silva et al., 1992a,b; Elgersma et al., 2002). Gene targeting was also used to create hippocampal area CA3-restricted null mutants of αCaMKII (Hinds et al., 2003). In addition, several lines of transgenic mice have been generated to increase αCaMKII activity levels, such as (1) transgenic Tg(T286D) mutants that have constitutive overexpression of mutated αCaMKII mimicking the autonomous activity of Thr^{286}-autophosphorylated kinase but being resistant to phosphatase regulation (Mayford et al., 1995), (2) inducible Tg(T286D) mutants, overexpressing the mutated αCaMKII protein under the control of the tet-OFF system (Mayford et al., 1996), and (3) Tg(F89G) mutants, overexpressing mutated αCaMKII protein having normal kinase activity but containing an altered structural domain allowing specific blockade by a designed inhibitor (Wang et al., 2003). The inhibitor blocks reversibly and does not inhibit endogenous αCaMKII.

A second group of mutants is aimed at investigating the physiological importance of αCaMKII autophosphorylation and of local αCaMKII protein synthesis. These mutants are generated by knock-in technology, resulting in a mutation of the endogenous αCaMKII gene. Mutants belonging to this group are (1) T286A mutants expressing αCaMKII that cannot be autophosphorylated at Thr^{286} (Giese et al., 1998), (2) T305V/T306A point mutants expressing αCaMKII protein that cannot be autophosphorylated at Thr^{305} and Thr^{306} (Elgersma et al., 2002), (3) T305D point mutants expressing αCaMKII mimicking constitutive autophosphorylation at Thr^{305} that blocks activation of the kinase by CaM (Elgersma et al., 2002), and (4) deletions of the dendritic localization signal within the 3′ untranslated region (UTR) of the αCaMKII mRNA that abrogate dendritic synthesis of CaMKIIα protein and reduce dendritic CaMKIIα protein levels (Miller et al., 2002).

Hippocampal synaptic plasticity

Induction and maintenance of long-term potentiation

The function of αCaMKII in synaptic plasticity has been most intensively studied at Schaffer collateral–CA1 pyramidal neuron synapses in the adult hippocampus. Analysis of the null mutants showed that the kinase is required for the induction of NMDA receptor-dependent long-term potentiation (LTP) at CA1 synapses (Silva et al., 1992a; Hinds et al., 1998; Elgersma et al., 2002). However, these studies also suggested that αCaMKII is not essential for CA1-LTP, because residual LTP could be obtained in the null mutants. Recent studies revealed a compensatory translocation of βCaMKII to PSDs (postsynaptic densities) in the null mutants, which is likely to be responsible for the residual LTP (Elgersma et al., 2002). No such compensation is observed in the T305D mutants and in the T286A mutants, and, in these mutants, CA1-LTP is completely absent (Giese et al., 1998; Elgersma et al., 2002). Because all βCaMKII subunits are associated with αCaMKII subunits, it is likely that these mutations in αCaMKII affect the function of the entire holoenzyme (Brocke et al., 1999).

Although αCaMKII and its autophosphorylation at Thr^{286} are

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Table 1. Overview of CaMKII mutants and their phenotypes

Mutant[a]	Purpose	Molecular characterization[b]	Plasticity[b]	Behavioral phenotype
αCaMKII-null (−/−)[1,2]	Elimination of αCaMKII protein	Expression level of βCaMKII is unaltered[1,2] but targeting of βCaMKII to PSD is increased[2]	HC-LTP reduced[1–3] NC-LTP reduced in adult animals[4–6] NC-LTP normal in young animals[4,5] Decreased PPF[1] Normal I/O[2]	Hippocampus-dependent learning severely impaired but learning in some animals is observed after extended training[1,2,6] Increased seizure susceptibility[7] (background dependent?[2])
αCaMKII-null (+/−)[1,2]	Reduction of αCaMKII protein	αCaMKII 50% reduced, βCaMKII levels normal	HC-LTP normal [2,8,9] NC-LTP reduced[9] Decreased PPF[8] Increased augmentation[8]	Normal learning[2] or reduced hippocampus-dependent learning[8] (background and protocol dependent) Severely impaired remote memory[10] Decreased fear and increased aggressive behavior[11]
αCaMKII-Floxed + CA3-cre[12]	Elimination of αCaMKII protein in CA3 area	αCaMKII is absent from CA3 area after 4 months of age	Increased frequency facilitation Normal PPF and basal P_r	Not tested
αCaMKII-T286A[13]	Blocks T286 phosphorylation (no autonomous activity)	Normal expression level of α/βCaMKII; Ca^{2+}-independent activity reduced by 60%	HC-LTP and NC-LTP impaired[13–16] Normal I/O[13]	Severely impaired learning in water maze[13,17] Unstable place cells[18]
Tg-αCaMKII-T286D[19]	Mimics constitutive T286 phosphorylation	Ca^{2+} independent activity two-fold increased	Favors LTD[19] Normal NC-LTP[20] Normal PPF[19]	Impaired learning in Barnes maze[21] Impaired olfactory-based spatial learning[22] Normal cued and contextual conditioning[21] Unstable place cells[23]
Tg-αCaMKII-T286D[24] (Tet inducible)	Mimics constitutive T286 phosphorylation in an inducible manner	Ca^{2+}-independent activity sixfold increased at maximum induction	Favors LTP at low expression level[25] Favors LTD at high expression level[24,25] NC-LTP and LTD normal[20]	Impaired learning in Barnes maze[24] Impaired learning in water maze[25] Impaired cued and contextual conditioning[24,25]
αCaMKII-TT305/6VA[2]	Blocks T305/T306 (inhibitory) phosphorylation	Increased levels of CaMKII in PSD	Favors LTP Normal LTD and I/O	Impaired water maze learning (initial learning okay) Impaired reversal learning Impaired context discrimination Old mice get seizures
αCaMKII-T305D[2]	Mimics constitutive T305 (inhibitory) phosphorylation	Severely reduced levels of α/βCaMKII in PSD	Favors LTD No LTP Normal LTD and I/O	Severely impaired in water maze and cued conditioning (cannot learn after overtraining)
αCaMKII-Δ3′UTR[26]	Impairs dendritic targeting of mRNA	Reduced amount of PSD associated CaMKII	Normal (early) LTP Reduced late-phase LTP	Impaired learning in water maze Normal STM but impaired LTM after cued and contextual conditioning
Tg-αCaMKII-F89G[27]	Inducible system allowing rapid (8 min) reversible decrease of αCaMKII transgene activity	Ca^{2+}-(in)dependent activity twofold to threefold increased without inhibitor	Normal I/O and PPF Enhanced LTD at 3 Hz Enhanced LTP at >10Hz	Impaired memory consolidation of contextual and cued conditioning when CaMKII activity is changed at the first week after training.
Ube3A[28,29]	Encodes E6-AP ubiquitin ligase; mouse model for Angelman's syndrome	Increased T286-P and T305-P[30] Decreased αCaMKII in PSD[30] Decreased PP1/PP2A activity[30]	Impaired LTP[28] Normal I/O[28]	Impaired spatial learning[28,29] Impaired motor coordination[28,29] Inducible seizures[28,29]

[a]References given in this column refer to primary papers describing the generation and analysis of the mutant. Mutants are made by homologous recombination unless otherwise specified.

[b]Molecular characterization and plasticity refers to the hippocampus unless otherwise specified.

[1]Silva et al., 1992; [2]Elgersma et al., 2002; [3]Hinds et al., 1998; [4]Glazewski et al., 1996; [5]Kirkwood et al., 1997; [6]Gordon et al., 1996; [7]Butler et al., 1995; [8]Silva et al., 1996; [9]Frankland et al., 2001; [10]Frankland et al., 2004; [11]Chen et al., 1994; [12]Hinds et al., 2003; [13]Giese et al., 1998; [14]Glazewski et al., 2000; [15]Hardingham et al., 2003; [16]Taha et al., 2002; [17]Need et al., 2003; [18]Cho et al., 1998; [19]Mayford et al., 1995; [20]Glazewski et al., 2001; [21]Bach et al., 1995; [22]Wiedenmayer et al., 2000; [23]Rotenberg et al., 1996; [24]Mayford et al., 1996; [25]Bejar et al., 2002; [26]Miller et al., 2002; [27]Wang et al., 2003; [28]Jiang et al., 1998; [29]Miura et al., 2002; [30]Weeber et al., 2003.

NC, Neocortex; I/O, input/output (a parameter used to assess synaptic transmission); HC, hippocampus; LTM, long-term memory (24 hr); PSD, postsynaptic density; PPF, paired pulse facilitation; P_r, probability of release; STM, short-term memory (30 min); Tg, transgenic.

essential for the induction of CA1-LTP, autophosphorylation at Thr^{286} is not needed for CA1-LTP during early postnatal life (Yasuda et al., 2003), which may explain the lack of morphological and developmental defects in the αCaMKII mutants.

αCaMKII is not only required for CA1-LTP induction but also plays a role in the late phase of LTP. Using mutant mice with a deletion of the 3′UTR in the αCaMKII mRNA, Miller et al. (2002) showed that these mutants are impaired in the protein synthesis-dependent phase of CA1-LTP. These data imply that late-phase LTP requires either local synthesis of new αCaMKII protein or some other important regulatory event abrogated by the constitutively reduced levels of dendritic αCaMKII protein.

Metaplasticity

αCaMKII appears to be a key player in the regulation of metaplasticity, which is defined as an activity-dependent change in synaptic plasticity and which controls the threshold of LTP induction to prevent saturation of synaptic plasticity (Abraham and Tate, 1997). In mice carrying the T305D mutation, which blocks activation and translocation of CaMKII to the PSD, a 10 Hz tetanus induces long-term depression (LTD), whereas this stimulus does not change synaptic efficacy in wild-type mice. In contrast, mice expressing αCaMKII, which cannot be phosphorylated at the inhibitory Thr^{305}/Thr^{306} sites, show marked LTP for the same stimulus (Elgersma et al., 2002). Importantly, although

T305D mice favor LTD and T305/T306VA mice favor LTP, a weak stimulus does not induce more LTD in T305D mutants, nor does a strong stimulus result in more LTP in T305/T306VA mice. This suggests that T305/T306 may be an important regulatory site in controlling the threshold of LTP induction. Furthermore, αCaMKII activity seems to lower the threshold of LTP induction. Consistent with this idea, a shift favoring LTP induction is observed in Tg(T286D) mutants, overexpressing low levels of constitutively active αCaMKII (Bejar et al., 2002). Moreover, the amount of αCaMKII can affect the amplitude of synaptic efficacy. Just increasing the total amount of αCaMKII protein gives rise to both increased LTD after weak stimulation and increased LTP after strong stimulation (Wang et al., 2003), thus exaggerating the bidirectional effects of αCaMKII on plasticity without changing the threshold in any particular direction. Together, these findings indicate that the phosphorylation (activation) state of CaMKII determines the threshold of LTP induction at weaker stimulation, whereas the total amount of available CaMKII determines the amplitude of the potentiation during very strong stimulation.

Clearly, there must be limits to the extent to which the threshold for LTD–LTP induction can be manipulated because it is essential for neuronal function to keep this window in a distinct working range. Indeed, high expression of constitutively active CaMKII in Tg(T286D) mutants shifts the threshold toward LTD rather than LTP (Mayford et al., 1995, 1996; Bejar et al., 2002). This is probably attributable to compensatory mechanisms that are recruited to deal with these high amounts of active CaMKII and that shift the threshold to the left. Currently, there is evidence for at least three such mechanisms. First, activated CaMKII expression can induce compensatory gene expression either in the neurons that express activated αCaMKII or other (inhibitory) neurons, such as an upregulation of the inhibitory neuropeptide Y, which could reduce the threshold of LTP induction (Bejar et al., 2002). Second, in *Drosophila*, it has been shown that neurons expressing activated CaMKII can decrease their intrinsic excitability by increasing potassium currents (Park et al., 2002). In addition, recent studies showed that expression of activated αCaMKII results in increased surface expression of $K_v4.2$ in cell culture, providing additional evidence that CaMKII can directly modulate intrinsic excitability (Varga et al., 2004). Third and finally, Turrigiano and colleagues demonstrated that postsynaptic expression of activated CaMKII in neuronal cultures leads to structural rearrangements that enhance connections from some presynaptic partners but eliminate connections from others (Pratt et al., 2003).

Presynaptic role

CaMKII also plays a role presynaptically. Measurements in the CA3-restricted null mutant indicate that presynaptic αCaMKII acts as an inhibitory constraint for neurotransmitter release, because these mice showed an enhancement of the activity-dependent increase in the probability of release (Hinds et al., 2003). In addition, heterozygous and homozygous null mutants show a remarkable increase of the augmentation phase (first few seconds) of the posttetanic potentiation (Chapman et al., 1995) and a decrease in paired-pulse facilitation (PPF) (Silva et al., 1992a; Chapman et al., 1995). Surprisingly, although changes in PPF are considered to reflect presynaptic changes, PPF was normal in the CA3-restricted null mutant (Hinds et al., 2003). To what extent the presynaptic changes contribute to the cognitive phenotype of the mutants remains to be elucidated.

Neocortical plasticity

Similar to CA1-LTP, the induction of LTP in the visual cortex and barrel cortex of adult animals was shown to depend on αCaMKII and its autophosphorylation at Thr^{286} (Glazewski et al., 1996, 2000; Gordon et al., 1996; Kirkwood et al., 1997; Taha et al., 2002; Hardingham et al., 2003). Neocortical LTP, however, is more sensitive to the amount of αCaMKII than CA1-LTP, because heterozygous null mutants show normal CA1-LTP (Chapman et al., 1995; Frankland et al., 2001; Elgersma et al., 2002) but a remarkable reduction of LTP in the neocortex (Frankland et al., 2001).

Interestingly, adult but not adolescent (1–2 months old) αCaMKII null mutants are impaired in neocortical plasticity (Glazewski et al., 1996; Kirkwood et al., 1997). The lack of an impairment in the adolescent null mutants is thought to be caused by a compensation mechanism, which does not occur in the T286A mutants at this age (Glazewski et al., 2000). These findings may be analogous to the findings in the hippocampus as mentioned above, in which it was shown that a translocation of βCaMKII can compensate for the loss of αCaMKII protein, but this compensation does not occur in the presence of mutated αCaMKII protein. Although autophosphorylation at Thr^{286} is essential for barrel cortex plasticity (Glazewski et al., 2000; Hardingham et al., 2003), overexpression of αCaMKII with the T286D mutation does not affect barrel cortex plasticity (Glazewski et al., 2001).

αCaMKII and cognition

Spatial learning

αCaMKII mutants have been studied primarily in hippocampus-dependent learning and memory tasks. Null mutants are severely impaired in spatial learning in the Morris water maze but show some learning after intensive training (Silva et al., 1992b; Elgersma et al., 2002). Similar to what has been found in the LTP studies, it is likely that a compensatory translocation of βCaMKII to the postsynaptic density is responsible for this residual spatial learning (Elgersma et al., 2002). In the T305D and the T286A mutants, in which no βCaMKII compensation occurs, a severe impairment was observed in spatial learning that cannot be rescued by overtraining (Giese et al., 1998; Elgersma et al., 2002). In principle, the spatial learning deficits could be caused by an interaction between the αCaMKII mutation and deprivation of the mice, which results from standard laboratory housing. However, environmental enrichment cannot rescue the spatial learning deficits in the T286A mutants (Need and Giese, 2003), indicating that the autophosphorylation of αCaMKII is essential for spatial learning.

Analysis of the T305/T306VA mutant has shown that inhibitory autophosphorylation is not strictly required for some spatial learning tasks but that phosphorylation of Thr^{305} is crucial for learning tasks that require flexible fine-tuning, such as reversal learning and contextual discrimination (Elgersma et al., 2002). For instance, T305/T306VA mutants can no longer find the platform in the water maze once it has been shifted to the opposite position in the pool, and they will show freezing in any chamber that has some similarity with the chamber in which the animals have been shocked.

Finally, analysis of the 3′UTR deletion mutants revealed learning deficits in the Morris water maze. Moreover, these mutants show normal fear conditioning after 30 min, whereas memory after 24 hr is impaired (Miller et al., 2002). Such a phenotype is consistent with the observed role of αCaMKII in the protein synthesis-dependent phase of late LTP (Miller et al., 2002).

Not only mutations in the endogenous αCaMKII gene impair

spatial learning, but also overexpression of αCaMKII affects learning in several hippocampus-dependent tasks, such as the Morris water maze, the Barnes maze, contextual conditioning, and novel object recognition (Bach et al., 1995; Mayford et al., 1996; Bejar et al., 2002; Wang et al., 2003). Because overexpression of αCaMKII^{T286D} can be induced after the development of the brain has been completed, these studies demonstrate a direct role for αCaMKII in learning (Bach et al., 1995; Mayford et al., 1996; Bejar et al., 2002; Wang et al., 2003).

How do mutations in αCaMKII affect spatial learning? Place cells are hippocampal pyramidal neurons that fire when the animal is in a particular location, and they are thought to be essential for spatial learning (O'Keefe and Dostrovsky, 1971). Analysis of place cell activity in the T286A mutants showed that the autophosphorylation at Thr^{286} is needed for spatial selectivity (signal-to-noise coding) and stability of place cells (Cho et al., 1998). Unstable place cells and impaired spatial learning in the Barnes maze were also found in the transgenic T286D mice expressing constitutive active αCaMKII (Bach et al., 1995; Rotenberg et al., 1996).

Memory

As mentioned above, plasticity of the neocortex seems more sensitive to reduced αCaMKII levels than hippocampal plasticity. This difference in sensitivity to αCaMKII levels probably underlies the remarkable phenotype of the heterozygous null mutants. These mutants are normal for recent memory after contextual conditioning or water maze training, but they are impaired in remote memory (>3 d after training) (Frankland et al., 2001). This finding was interesting because the hippocampus is thought to play only a temporary role in storage of contextual fear memory. Lesion studies have shown that the hippocampus is not required for contextual memory storage 28 d after training (Kim and Fanselow, 1992). Therefore, it has been suggested that contextual memory is transferred from the hippocampus to neocortex, and αCaMKII seems to contribute to this transfer. Consistent with this idea, the activation of immediate-early gene expression in neocortex is impaired in the αCaMKII heterozygotes after contextual fear conditioning (Frankland et al., 2004).

A direct role for αCaMKII in memory consolidation and/or retrieval was demonstrated using mice with an inducible T286D transgene. Activation of the transgene after learning resulted in impaired recall 6 weeks later (Mayford et al., 1996). The temporary contribution to memory consolidation could be tested more precisely using Tg(F89G) mutants. The αCaMKII^{F89G} contains an altered structural domain allowing specific and rapid (8 min after injection) blockade by a designed inhibitor in which the activity of the overexpressed αCaMKII^{F89G} can be downregulated. Increasing αCaMKII activity 1–7 d after training affected the recall test 30 d later, whereas no effects were observed when αCaMKII activity was changed between days 7 and 28 after training (Wang et al., 2003). This indicates that αCaMKII plays a critical role in memory consolidation during the first days after training. Furthermore, it was shown that αCaMKII is involved in memory recall, because the transgenic mice showed significantly less freezing in both contextual and cued freezing tested at either 1 d retention or 1 month retention when overexpressed αCaMKII^{F89G} was not inhibited at the time of testing (Wang et al., 2003).

Other phenotypes

Because αCaMKII is expressed in most brain areas, one could expect this kinase to be involved in all types of learning. However, normal learning was shown in several non-hippocampus-dependent learning tasks, such as the visible platform water maze, plus maze, cued Barnes maze, olfactory discrimination, the acquisition of instrumental conditioning, and the accelerating rotarod (Silva et al., 1992b; Bach et al., 1995; Giese et al., 1998; Wiedenmayer et al., 2000; Carvalho et al., 2001; Elgersma et al., 2002).

Besides the almost surprising specificity of the learning impairments, a few other neurological phenotypes have been reported. Some of these behaviors (e.g., increased activity in an open field and Y-maze) parallel responses seen in hippocampus-lesioned animals (Silva et al., 1992b). Also, the increased seizure susceptibility in some αCaMKII mutants (Butler et al., 1995; Elgersma et al., 2002) is likely to be associated with changes in hippocampal plasticity or could result from changes in intrinsic excitability. Another behavioral phenotype that has been observed is an abnormal fear response and concomitant increased aggression of the heterozygous null mutants (Chen et al., 1994), which severely complicates breeding of the animals. Because the phenotype has become progressively worse over the course of inbreeding and because it is not observed (yet?) in the recently developed knock-out strain (Elgersma et al., 2002), this phenotype is likely to be dependent on the genetic background of the animals. Abnormal fear responses may also explain deficits in the cued conditioning task. To our knowledge, this is up to now the only reported non-hippocampus-dependent learning task in which most of the αCaMKII mutants are shown to be impaired (Silva et al., 1992b, 1996; Bach et al., 1995; Mayford et al., 1996; Bejar et al., 2002; Miller et al., 2002; Wang et al., 2003).

Human learning and memory

To date, no mutations have been identified in the human αCaMKII gene that could underlie a genetic neurological disease. However, recent findings suggest that abnormal αCaMKII activity is likely to contribute to the symptoms of AS. AS is a form of mental retardation associated with profound cognitive dysfunction, including severe learning deficits and an associated ataxia (Clayton-Smith and Laan, 2003; Guerrini et al., 2003). The human AS gene encodes an ubiquitin ligase: specifically the UBE3A (i.e., E6-AP) ubiquitin ligase that transfers ubiquitin moieties to a restricted set of substrate proteins (Matsuura et al., 1997; Clayton-Smith and Laan, 2003; Guerrini et al., 2003). This information was used by two groups to independently generate two mouse models of AS (Jiang et al., 1998; Miura et al., 2002). Using these mouse models, both groups found that the *Ube3a* gene is imprinted with silencing of the paternal allele in hippocampus and cerebellum in mice, as it is in humans (Albrecht et al., 1997). In other words, the product of the maternal *Ube3a* gene is selectively absent in the hippocampus, olfactory bulb, and cerebellum in human AS and in the mouse AS model. The behavioral phenotype of mice with maternal deficiency (m^-/p^+) for *Ube3a* resembles human Angelman's syndrome, manifesting motor dysfunction, inducible seizures, and a context-dependent associative learning deficit (Jiang et al., 1998; Miura et al., 2002). CA1-LTP is also severely impaired in m^-/p^+ mice, despite normal baseline synaptic transmission and neuroanatomy, indicating that ubiquitination plays a role in regulating mammalian LTP (Jiang et al., 1998; Weeber et al., 2003). Physiological studies of synaptic plasticity in the AS model mouse hippocampal slices *in vitro* indicate that the AS-associated defects in the mouse model appear to be downstream of postsynaptic calcium influx, implicating known targets of calcium signaling as potential mediators of dysfunction (Weeber et al., 2003). Significantly, AS model mutant animals exhibit a significant increase in hippocampal phospho-CaMKII, specifically at sites Thr^{286} and Thr^{305}, with no corresponding change in the levels of total CaMKII. In addition,

these mutants show reduced CaMKII enzymatic activity and decreased *post hoc* autophosphorylation capability (Weeber et al., 2003). Interestingly, despite its increased phosphorylation at Thr^{286}, there is less CaMKII associated with the postsynaptic density, providing additional evidence that phosphorylation at Thr^{305} reduces the affinity for the postsynaptic density (Elgersma et al., 2002; Weeber et al., 2003). Because this phenotype shows a remarkable similarity with the CaMKII mutants mimicking persistent Thr^{305} phosphorylation (Elgersma et al., 2002), these findings suggest that misregulation of CaMKII phosphorylation may account for many of the synaptic plasticity deficits and behavioral deficits in the mouse model for AS (Weeber et al., 2003). The misregulation of CaMKII dephosphorylation is probably caused by the decreased protein phosphatase PP1/PP2A activity in this mutant based on *in vitro* studies of phosphatase activity in the AS model mice (Weeber et al., 2003). Although both phosphatases have been directly implicated in CaMKII regulation (Colbran, 2004), the linkage between the UBE3A gene product and regulation of phosphatase activity is completely mysterious at this point.

Conclusion

Mouse genetic approaches have provided us with a wealth of information on the role of αCaMKII in plasticity and cognition. These studies would not have been possible without the overwhelming amount of data gathered from biochemical and cellular studies over the last two decades. Conversely, without the mutants, the physiological relevance of these *in vitro* findings could not have been revealed. αCaMKII mutants have also proven to be a useful tool to unravel the temporal aspects of memory acquisition, consolidation, and retrieval. Studies on αCaMKII mutants have been one of the leading areas in investigating the molecular basis of synaptic plasticity historically, and there are exciting times ahead as investigations in this area continue.

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