

5. Spaink, H. P., Kondorosi, A. & Hooykaas, P. J. J. (eds) *The Rhizobiaceae* (Kluwer, Dordrecht, 1998).
6. Long, S. R. *Rhizobium* symbiosis: Nod factors in perspective. *Plant Cell* **8**, 1885–1898 (1996).
7. López-Lara, I. M. *et al.* Structural identification of the lipo-chitin oligosaccharide nodulation signals of *Rhizobium loti*. *Mol. Microbiol.* **15**, 627–638 (1995).
8. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. The leucine zipper: A hypothetical structure common to a new class of DNA binding proteins. *Science* **240**, 1759–1764 (1988).
9. Ferré-D'Amaré, A. R., Prendergast, G. C., Ziff, E. B. & Burley, S. K. Recognition by Max of its cognate DNA through a dimeric b/HLH/Z domain. *Nature* **363**, 38–45 (1993).
10. Puyet, A., Ibanez, A. M. & Espinosa, M. Characterisation of the *Streptococcus pneumoniae* maltosaccharide MalR, a member of the LacI-GalR family of repressors displaying distinctive genetic features. *J. Biol. Chem.* **268**, 25402–25408 (1993).
11. Silver, P. A. How proteins enter the nucleus. *Cell* **64**, 489–497 (1991).
12. Plano, G. V. & Winkler, H. H. Identification and initial topological analysis of the *Rickettsia prowazekii* ATP/ADP translocase. *J. Bacteriol.* **173**, 3389–3396 (1991).
13. Lehmbek, J. *et al.* Sequence of two genes in pea chloroplast DNA coding for 84 and 82 kD polypeptides of the photosystem I complex. *Plant Mol. Biol.* **7**, 3–10 (1986).
14. Ferris, P. J. & Goodenough, U. W. Mating type in *Chlamydomonas* is specified by *mid*, the Minus-dominance gene. *Genetics* **146**, 859–869 (1997).
15. Ferris, P. J., Pavlovic, C., Fabry, S. & Goodenough, U. W. Rapid evolution of sex-related genes in *Chlamydomonas*. *Proc. Natl Acad. Sci. USA* **94**, 8634–8639 (1997).
16. Beck, C. F. & Haring, M. A. Gametic differentiation of *Chlamydomonas*. *Int. Rev. Cytol.* **168**, 259–302 (1996).
17. Yokoyama, C. *et al.* SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. *Cell* **75**, 187–197 (1993).
18. Brown, M. S. & Goldstein, J. L. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell* **89**, 331–340 (1997).
19. Sakai, J. *et al.* Molecular identification of the sterol-regulated luminal protease that cleaves SREBPs and controls lipid composition of animal cells. *Mol. Cell* **2**, 505–514 (1998).
20. Schroeter, E. H., Kisslinger, J. A. & Kopan, R. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature* **393**, 382–386 (1998).
21. Thykjaer, T., Stiller, J., Handberg, K., Jones, J. & Stougaard, J. The maize transposable element *Ac* is mobile in the legume *Lotus japonicus*. *Plant Mol. Biol.* **27**, 981–993 (1995).
22. Schaefer, L. *et al.* Symbiotic mutants deficient in nodule establishment identified after T-DNA mutagenesis of *Lotus japonicus*. *Mol. Gen. Genet.* **259**, 414–423 (1998).
23. Sullivan, J. T. *et al.* Nodulating strains of *Rhizobium loti* arise through chromosomal symbiotic gene transfer in the environment. *Proc. Natl Acad. Sci. USA* **92**, 8985–8989 (1995).
24. Boivin, C. *et al.* *Rhizobium melliloti* genes encoding catabolism of trigonelline are induced under symbiotic conditions. *Plant Cell* **2**, 1157–1170 (1990).
25. Earp, D. J., Lowe, B. & Baker, B. Amplification of genomic sequences flanking transposable elements in host and heterologous plants: a tool for transposon tagging and genome characterization. *Nucleic Acids Res.* **18**, 3271–3279 (1990).
26. Papadopoulou, K., Roussis, A. & Katinakis, P. Phaseolus ENOD40 is involved in symbiotic and non-symbiotic organogenetic processes: expression during nodule and lateral root development. *Plant Mol. Biol.* **30**, 403–417 (1996).

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Correspondence and requests for materials should be addressed to J.S. (e-mail: stougaard@mbio.aau.dk). The *Nin* gene sequence and cDNA sequence have been deposited in the EMBL database (accession nos AJ238956 and AJ239041, respectively).

CaMKII regulates the density of central glutamatergic synapses *in vivo*

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Synaptic connections undergo a dynamic process of stabilization or elimination during development, and this process is thought to be critical in memory and learning and in establishing the specificity of synaptic connections¹. The type II calcium- and calmodulin-dependent protein kinase (CaMKII) has been proposed to be pivotal in regulating synaptic strength^{2–4} and in maturation of synapses during development⁵. Here we describe how CaMKII regulates the formation of central glutamatergic synapses in *Caenorhabditis elegans*. During larval development, the density of ventral nerve cord synapses containing the GLR-1

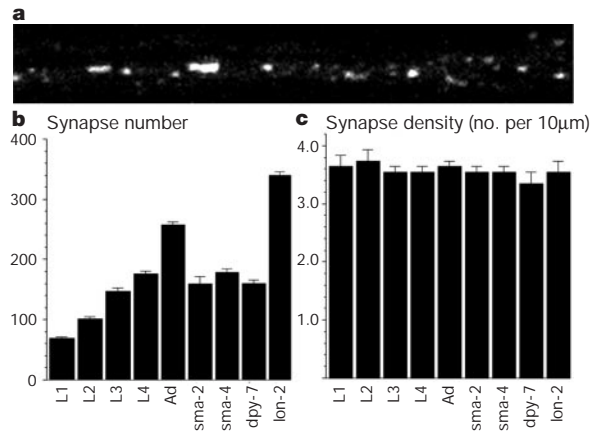


Figure 1 Formation of GLR-1::GFP clusters. **a**, GLR-1::GFP accumulates in clusters along the lengths of neurites in the ventral nerve cord. **b**, Individual GLR-1::GFP clusters in the ventral nerve cord were counted in wild-type larvae (L1–L4) and adults (Ad), as well as in mutant adults with short (*sma-2*, *sma-4* and *dpy-7*) or long (*lon-2*) body lengths. **c**, Data plotted as synaptic density (number of GLR-1::GFP clusters per 10 μm nerve cord length). Values are means ± s.e.m. Ten to thirty animals were examined for each entry.

glutamate receptor is held constant despite marked changes in neurite length. The coupling of synapse number to neurite length requires both CaMKII and voltage-gated calcium channels. CaMKII regulates GLR-1 by at least two distinct mechanisms: regulating transport of GLR-1 from cell bodies to neurites; and regulating the addition or maintenance of GLR-1 to postsynaptic elements.

To determine how central synapses change in number and morphology during development, we observed neuron–neuron synapses that contain the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)-type glutamate receptor (GluR) GLR-1 (refs 6, 7). We previously showed that chimaeric receptors tagged with the green fluorescent protein (GLR-1::GFP) can be used to visualize central glutamatergic synapses in living animals⁸. GLR-1::GFP is localized to discrete punctate structures (Fig. 1a; hereafter called clusters) along the lengths of neurites in both the ventral cord and nerve ring processes, where the majority of interneuron synapses reside⁹. GLR-1-containing neurites extend the full length of the ventral nerve cord throughout larval and adult development; thus, these neurites must elongate as the animal grows⁹. To see how synapse numbers are affected by changes in neurite length, we examined the number of GLR-1::GFP clusters in the ventral cord during larval development (Fig. 1b). First stage (L1) larvae had ~60 clusters whereas adults had ~260 clusters in the ventral cord. This apparent increase in GLR-1-containing synapses occurs despite the fact that the number of GLR-1-expressing cells remains constant throughout larval development^{6,7}. Homozygous *lin-5* mutants, which lack postembryonic cell divisions¹⁰, have similar numbers of GLR-1::GFP clusters (193 ± 17, *n* = 10) as their age-matched (L4 stage) heterozygous siblings (181 ± 6, *n* = 10). Therefore, the GLR-1::GFP clusters added during larval development are not induced by synaptic partners produced by post-embryonic lineages. One simple model explaining these results is that developing animals maintain a constant density of GLR-1-containing synapses along the ventral cord, thereby necessitating addition of synapses to compensate for increased body length.

We tested the relationship between synapse number and body length by comparing the number of GLR-1::GFP clusters with the length of the ventral cord in developing worms. Although cluster number varies nearly fourfold from early L1 to adulthood, the density of clusters was constant throughout larval development (~3.7 per 10 μm) (Fig. 1b, c). Similarly, compensatory changes occurred in the number of GLR-1::GFP clusters in short and long

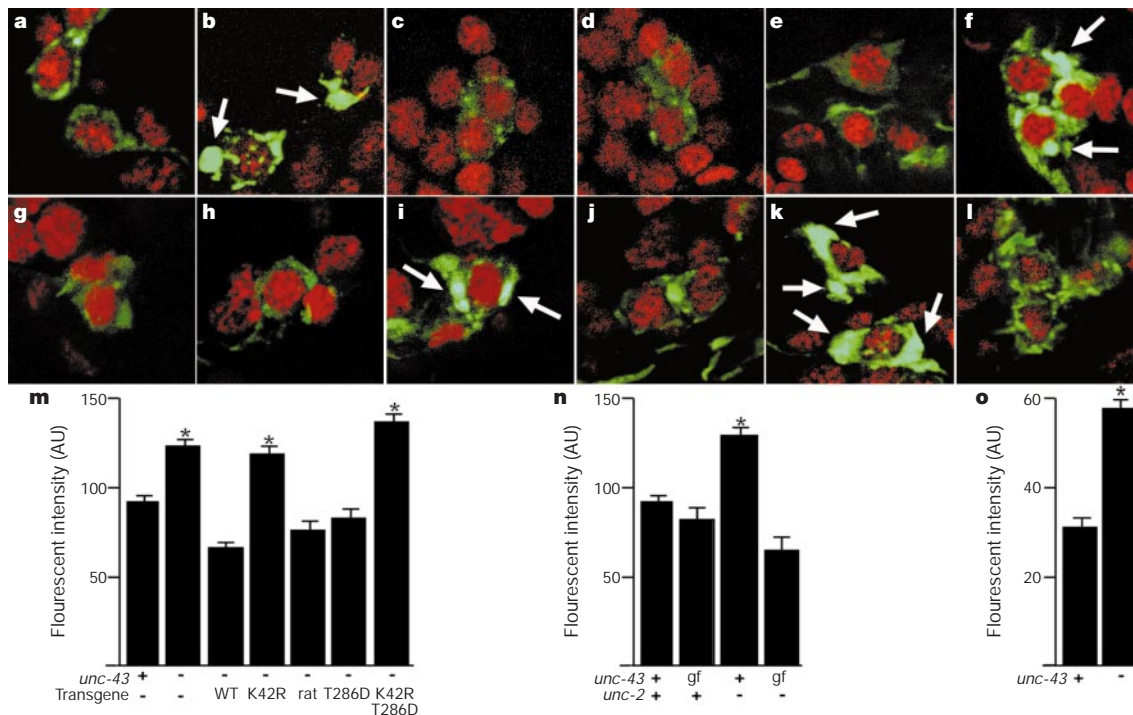


Figure 2 Mutants lacking the *unc-43* CaMKII accumulate GLR-1::GFP receptors in neuron cell bodies. Accumulation of GLR-1::GFP (a, b, e–l) or SNB-1::GFP (c, d), both shown in green, in the cell bodies of GLR-1-expressing interneurons was compared in various strains. Nuclei are labelled with propidium iodide (red). a, Wild-type. b, Homozygous *unc-43(lf)* CaMKII mutants carrying either no transgene, or *glr-1* promoted transgenes containing a wild-type UNC-43 cDNA (e), a kinase-defective mutant UNC-43(K42R) cDNA (f), a wild-type rat α CaMKII cDNA (g), a constitutively active UNC-43(T286D) cDNA (h), or a double mutant UNC-43(K42R, T286D) cDNA (i). j, Homozygous *unc-43(gf)* CaMKII

mutants. k, Homozygous *unc-2(lf)* calcium-channel mutants. l, *unc-43(gf)* CaMKII; *unc-2(lf)* double mutants. For comparison, equivalent amounts of SNB-1::GFP were found in the cell bodies of wild-type (c) and homozygous *unc-43(lf)* CaMKII mutant (d) interneurons. GLR-1::GFP (m, n) or endogenous GLR-1 (o) (detected by anti-GLR-1 antibodies²) cell-body fluorescence was quantified by measuring the pixel intensity of confocal projections. Values shown are means \pm sem (AU, arbitrary units). From 19 to 55 nuclei were examined for each genotype. Asterisk, values significantly different ($P \leq 0.0001$) from wild type.

Table 1 UNC-43 regulates GLR-1 synaptic density

Effect of Ca ²⁺ and neural activity on GLR-1 synaptic density†	Gene product	Synapse density (no. per 10 μ m)
Wild type		3.7 \pm 0.1
Synaptic transmission		
<i>unc-13</i>	Munc13	4.1 \pm 0.1
<i>unc-18</i>	Sec1p	3.7 \pm 0.1
<i>unc-64</i>	Syntaxin	3.8 \pm 0.1
<i>unc-104</i>	SV Kinesin	4.0 \pm 0.1
<i>snt-1</i>	Synaptotagmin	4.1 \pm 0.1
Ca ²⁺ signalling		
<i>unc-43</i>	α CaMKII	2.3 \pm 0.1*
<i>unc-2</i>	VDCC α 1	2.5 \pm 0.1*
<i>unc-36</i>	VDCC α 2	2.8 \pm 0.1*
<i>egl-19</i>	VDCC α 1	2.0 \pm 0.1*
<i>egl-19(gf)</i>		2.7 \pm 0.1*
<i>unc-2; egl-19</i>		2.3 \pm 0.1*
<i>unc-2; unc-43</i>		2.2 \pm 0.1*
UNC-43 regulates GLR-1 synaptic density		
Genotype	Transgene‡	Synapse density (no. per 10 μ m)
Wild type	–	3.7 \pm 0.1
<i>unc-43</i>	–	2.4 \pm 0.1*
<i>unc-43</i>	WT	3.6 \pm 0.1
<i>unc-43</i>	K42R	2.5 \pm 0.1*
<i>unc-43</i>	rat α	3.5 \pm 0.1
<i>unc-43(gf)</i>	–	2.7 \pm 0.1*
<i>unc-43</i>	T286D	3.1 \pm 0.1*
<i>unc-43</i>	K42R, T286D	2.4 \pm 0.1*
<i>unc-2</i>	–	2.5 \pm 0.1*
<i>unc-2; unc-43(gf)</i>	–	2.1 \pm 0.1*

Mutations are loss of function, unless gain of function is specified (gf). From 10 to 30 animals were examined for each genotype. Values are means \pm s.e.m.

* Indicates a significant difference ($P < 0.0001$, t-test) from wild type.

† Synaptic density (number of GLR-1::GFP clusters per 10 μ m length of ventral nerve cord) was counted for each genotype.

‡ Transgene indicates the version of *unc-43* or rat CaMKII cDNA expressed by the *glr-1* promoter in the indicated genetic background. SV, synaptic vesicle; VDCC, voltage-dependent calcium channel; WT, wild type.

mutant adults, resulting in a constant density despite greatly differing adult body lengths (Fig. 1b, c). These results reveal a homeostatic mechanism coupling GLR-1::GFP synapse numbers and body length. A similar coupling of synaptic growth to increased size of the synaptic target is also seen at mouse and fly neuromuscular junctions¹¹. This phenomenon has been proposed as a mechanism to maintain relatively constant levels of postsynaptic excitation during developmental growth.

Are changes in synaptic transmission required to promote the increased number of GLR-1 synapses formed during development? We tested this idea by examining GLR-1::GFP clusters in mutants that have greatly reduced release of neurotransmitter, including *unc-13*, *unc-18* (nSec-1), *unc-64* (syntaxin), *snt-1* (synaptotagmin) and *unc-104* (kinesin)¹². None of these mutations reduced the number of GLR-1::GFP clusters in L1 larvae or young adults (Table 1). In fact, a modest increase in the density of GLR-1::GFP clusters was observed in some cases. These results are consistent with reports that a blockade of synaptic transmission often increases the number of postsynaptic elements in cultured rodent neurons^{13,14}.

CaMKII has been proposed to act as a critical signalling element regulating changes in synaptic strength in vertebrates^{2–4}. The *C. elegans* genome contains a single CaMKII gene, which corresponds to the *unc-43* gene¹⁵. Loss-of-function mutations in *unc-43* (*unc-43(lf)* alleles) reduce CaMKII activity, whereas a gain-of-function mutation (*unc-43(gf)*) causes constitutive calcium-independent activity¹⁵. Both *unc-43(lf)* and *unc-43(gf)* mutants had a significantly reduced density of clusters in adults (Table 1) but not in L1 larvae (data not shown). The cluster density in *unc-43(lf)* mutants was restored to wild-type levels by expressing a wild-

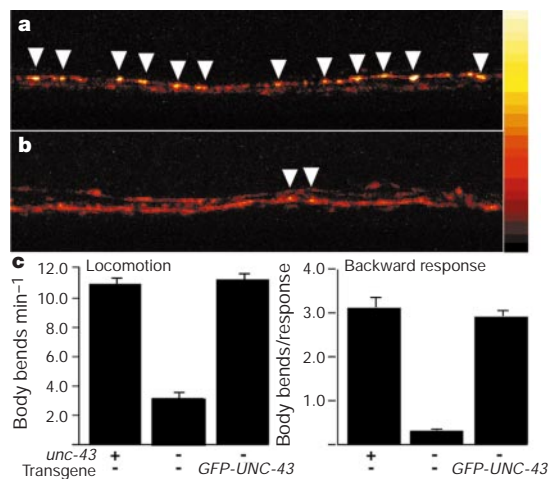


Figure 3 GFP::UNC-43 is localized to clusters in the ventral cord processes. **a, b**, Grey scale GFP::UNC-43 images were converted into colour according to the colour look-up table shown on the right. **a**, Wild-type neurons that express GFP::UNC-43 contain GFP::UNC-43 in clusters (arrowheads) in their neural processes. **b**, Wild-type neurons that express GFP::UNC-43 with a T286D substitution (GFP::UNC-43(T286D)) contain diffuse GFP::UNC-43(T286D) with few GFP::UNC-43(T286D) clusters (arrowheads) in their neural processes. **c**, Locomotion (worm body bends per min) and backward response to anterior body touch (worm body bends per touch) were counted for each genotype after a 1-h heat shock (33 °C). Transgene indicates whether *HS-GFP-unc-43* (GFP::UNC-43 expressed by the heat-shock promoter) was in the indicated genetic background.

type UNC-43 cDNA in the GLR-1-containing cells, whereas expression of a catalytically inactive mutant, UNC-43 (K42R), had no effect. Expression of a constitutively active, calcium-independent form of CaMKII, UNC-43(T286D)¹⁶, in the GLR-1-expressing interneurons also produced a significant reduction in the density of GLR-1::GFP clusters, whereas expression of the kinase-dead double mutant, UNC-43(K42R,T286D), did not (Table 1). These results indicate that CaMKII activity in GLR-1-expressing cells may regulate the addition of GLR-1-containing synapses in the ventral cord during larval growth.

What is the source of calcium that activates CaMKII? The *unc-2*, *unc-36* and *egl-19* genes encode subunits of voltage-dependent calcium channels^{17,18}. We found that loss-of-function mutations in *unc-2* and *egl-19* (and to a lesser extent *unc-36*) produced significant reductions in GLR-1::GFP clusters in adults (Table 1). Moreover, a gain-of-function mutation that is predicted to increase calcium influx, *egl-19(n2368sd)*¹⁸, also significantly reduced the density of GLR-1::GFP clusters in adults, similar to the defect seen in *unc-43(gf)* and *unc-43(T286D)* mutants. These results indicate that calcium influx through channels containing EGL-19 and UNC-2 subunits may regulate formation of GLR-1::GFP clusters in the ventral cord. Although both regulate cluster density, the calcium channels and *unc-43* CaMKII may regulate GLR-1 by different mechanisms. If this were the case, we would expect that double mutants lacking both CaMKII and the calcium channels would have more severe defects in cluster density than either single mutant. In contrast, we found that the cluster densities of the *unc-43(lf)*; *unc-2(lf)* double mutant and single mutants were indistinguishable (Table 1). These results indicate that the calcium channels and CaMKII form a single pathway regulating the density of GLR-1 synapses, as expected if calcium influx through these channels activates the endogenous *unc-43* CaMKII.

The effects of the *unc-43* and the calcium-channel mutations are both consistent with a model in which CaMKII plays both stimulatory and inhibitory roles in GLR-1 synapse formation. On the one hand, CaMKII activity promotes synapse formation, as seen by the decreased density of GLR-1::GFP clusters in mutants with either decreased calcium influx or decreased *unc-43* CaMKII activity. On

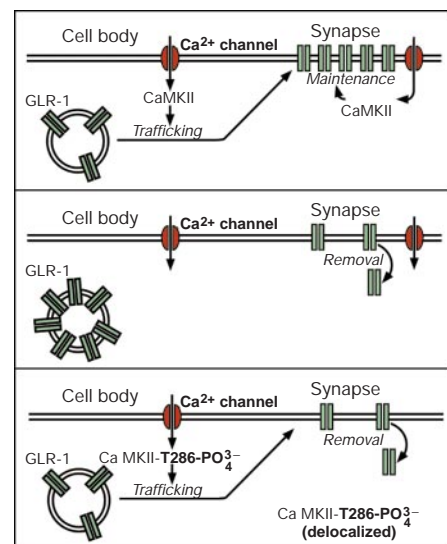


Figure 4 A model for CaMKII regulation of synaptic density. **a**, In wild-type worms, calcium influx through voltage-gated calcium channels and CaMKII activity promote transport of GLR-1 from the cell body to synapses, and addition or stability of GLR-1 at postsynaptic elements. **b**, In worms that lack *unc-43* CaMKII activity, GLR-1 accumulates in the cell body. **c**, Mutations that make *unc-43* CaMKII constitutively active result in proper trafficking of GLR-1, but fail to add or maintain GLR-1 at synapses, probably because the constitutively active form of CaMKII is not localized to synapses.

the other hand, CaMKII can also inhibit synapse formation, as seen by the decreased density of clusters in mutants with increased calcium influx or constitutive *unc-43* CaMKII activity. Although the underlying mechanisms might differ, there are precedents for both potentiation and depression of synaptic strength by CaMKII in vertebrates^{2,3}. CaMKII regulates growth of dendrites¹⁹; however, we found that the length of the interneuron neurites was not altered by changes in *unc-43* CaMKII activity (data not shown).

As a first step toward determining how *unc-43* CaMKII regulates the formation of GLR-1 synapses, we compared the subcellular localization patterns of GLR-1::GFP with a rescuing full-length GFP-tagged UNC-43 reporter (GFP::UNC-43)⁸. Both GFP-fusion proteins were found in ventral cord clusters, as well as in perinuclear structures in cell bodies (Figs 2a and 3a; and data not shown). Thus, *unc-43* CaMKII could regulate GLR-1::GFP in processes occurring either in the cell body (for example, intracellular trafficking) or in the axon (for example, addition of receptors to postsynaptic elements). The perinuclear GLR-1::GFP (Fig. 2a) is probably an intermediate in transport of GLR-1 to the axons, as transiently expressed GLR-1::GFP (utilizing a heat-shock vector) accumulated in this compartment at early times after heat-shock, but was found in axons at later time points (data not shown).

Mutants lacking either *unc-43* CaMKII or the calcium channels (*unc-2* and *egl-19*) accumulated high levels of GLR-1::GFP in the cell body, particularly in these perinuclear structures (Fig. 2b, k; and data not shown); however, the amount of GLR-1::GFP in individual ventral cord clusters of these mutants did not differ from that in wild-type animals (data not shown). Qualitatively similar results were observed when we compared the distribution of endogenously expressed GLR-1 (stained with anti-GLR-1 antibodies, Fig. 2o). Other synaptic proteins (for example, the vesicle protein SNB-1 (ref. 20)) did not accumulate in the cell bodies of *unc-43* mutants (82 ± 6 arbitrary fluorescence units (AU)), *n* = 25, compared with 98 ± 7 AU, *n* = 33 for wild type), indicating that CaMKII does not globally regulate the trafficking of neuronal membrane proteins (Fig. 2c, d). Expression of a wild-type UNC-43 complementary DNA in the GLR-1-expressing neurons rescued both the GLR-1 trafficking and the decreased synaptic-density defects, whereas

expression of the kinase-dead UNC-43(K42R) cDNA rescued neither (Table 1; Fig. 2e, f). Similarly, expression of a rat α CaMKII cDNA in the GLR-1-expressing neurons also rescued both the lowered synaptic density and the trafficking defects of *unc-43* mutants (Table 1; Fig. 2g), showing that this function of CaMKII has been conserved across phylogeny. Our results indicate that *unc-43* CaMKII regulates the trafficking of GLR-1 from the cell body to neurites and that CaMKII may possibly regulate the trafficking of AMPA receptors in mammalian neurons. These results are also consistent with reports that the delivery of AMPA receptors to postsynaptic elements in rodent neurons is regulated by neural activity^{13,14,21–23}.

Constitutive activation of *unc-43* CaMKII also resulted in reduced numbers of GLR-1-synapses; however, in this case, GLR-1::GFP did not accumulate in the cell bodies of neurons (Fig. 2h, j). The GLR-1 trafficking defect found in calcium-channel mutants was not observed in *unc-43(gf); unc-2(lf)* double mutants (Fig. 2l), as expected if calcium influx through these channels activates *unc-43* CaMKII. These results also indicate that the normal calcium-dependent form of CaMKII activity (in wild-type animals) may have different physiological effects from the constitutively active version (which reduces synapse density). One model that might explain this is that these two forms of CaMKII are localized differently within neurons, and hence they have distinct effects. A GFP-tagged version of rat α CaMKII has been reported to be properly localized to synapses in cultured neurons²⁴, and we constructed a similar GFP-tagged form of UNC-43 to monitor its subcellular distribution *in vivo*. Expression of the GFP::UNC-43 construct corrected two behavioural defects observed in homozygous *unc-43(lf)* mutants (Fig. 3c), suggesting that fusion to the GFP moiety did not perturb the function or subcellular localization of *unc-43* CaMKII. Wild-type GFP::UNC-43 was localized in ventral cord clusters (Fig. 3a; 3.7 ± 0.4 clusters per $10 \mu\text{m}$, $n = 13$). In contrast, GFP::UNC-43(T286D) was diffusely distributed and had significantly ($P = 0.0001$) fewer ventral cord clusters (Fig. 3b; 1.3 ± 0.2 clusters per $10 \mu\text{m}$, $n = 11$). These two transgenes were expressed at similar levels (28 ± 3 AU, $n = 23$ for GFP::UNC-43, and 31 ± 3 AU, $n = 24$ for GFP::UNC-43(T286D)), indicating that the difference in localization pattern was not due to differences in expression levels. The *unc-43* CaMKII activity was not required for localization, as the kinase-dead GFP::UNC-43(K42R) protein was localized to clusters whereas a kinase-dead double mutant GFP::UNC-43(K42R, T286D) was not (data not shown). Thus, constitutively-activated CaMKII fails to localize to synaptic sites and also decreases the density of GLR-1::GFP clusters. These results indicate that synaptically localized calcium-dependent CaMKII activity may be required for either addition or maintenance of GLR-1::GFP at synaptic sites (Fig. 4a, b). Upon constitutive activation in response to high calcium levels, CaMKII is removed from synaptic sites and can no longer add or maintain GLR-1::GFP at these sites (Fig. 4c). These results are consistent with reports that synaptic localization of rat α CaMKII changes in response to autophosphorylation²⁴.

We have shown that CaMKII and voltage-dependent calcium channels are required for the homeostatic control of synaptic density during growth. We propose that this regulation is mediated by two distinct antagonistic functions of CaMKII (Fig. 4). As the worm grows in size, the synaptic density along the ventral cord drops, which should result in reduced excitation of the interneurons. Under these conditions, CaMKII induces formation of new synapses, perhaps by augmenting transport of nascent receptors from the cell bodies. Consistent with this idea, a slightly increased density of GLR-1::GFP clusters occurred in mutants that have reduced synaptic transmission, and hence should mimic reduced synaptic density (Table 1). Conversely, too high a synaptic density would cause increased excitation and constitutive CaMKII activity, which would destabilize existing synapses or prevent the

addition of new ones. One caveat for our results is that we have not directly measured changes in CaMKII activity in mutant or transgenic neurons. Thus, we cannot state unequivocally that the *unc-43(gf)* mutations or transgenes are actually changing the total kinase activity in these cells. Nonetheless, it is clear that *unc-43* CaMKII catalytic activity is required for maintaining the proper GLR-1 synaptic density and for GLR-1 trafficking. The mechanism by which CaMKII regulates GLR-1 trafficking and localization is not known but might involve direct phosphorylation of GLR-1^{25,26}.

Our results are similar in some respects to a form of plasticity, synaptic scaling^{11,27}. The synapses of cultured neurons are scaled up or down together, in response to changes in neural activity. Likewise, we have found that interneurons *in vivo* increase or decrease the number of postsynaptic elements, depending on their pattern of activity. Our results indicate that phenomena similar to synaptic scaling may occur *in vivo* and may be utilized to maintain constant density of excitatory synapses in response to dramatic growth of axons during development. □

Methods

Strains

dpy-7(e88), *egl-19(n582,ad695sd,n2368sd)*, *lin-5(e1348)*, *lon-2(e678)*, *sma-2(e502)*, *sma-4(e729)*, *snt-1(n2665)*, *unc-2(e55,e97,e129)*, *unc-13(e51)*, *unc-18(e81)*, *unc-36(e251)*, *unc-43(n1186,e408,n498sd)*, *unc-64(e246)* and *unc-104(e1265,rh142)*.

Molecular biology

The rat α CaMKII transgene (KP#350) was generated by ligating the rat α CaMKII cDNA (M. Kennedy) into the pV6 vector (V. Maricq), which contains the *glr-1* promoter and the *unc-54* 3' UTR. The *unc-43(T286D)* (KP#285), *unc-43(K42R)* (KP#351) and *unc-43(K42R, T286D)* (KP#352) transgenes were generated by modifying the *unc-43* cDNA by PCR, and verified by sequencing. The *GFP-unc-43(T286D)* (KP#287) transgene was generated by ligating the *unc-43(T286D)* cDNA into KP#241, a vector containing the *glr-1* promoter, a synthetic intron, GFP, and the *unc-54* 3' UTR. The *HS-GFP-unc-43* (KP#353) transgene was generated by ligating *GFP-unc-43* into PD49.78 (A. Fire).

Expression of transgenes

Transgenic strains were isolated by micro-injecting plasmids (typically at $50 \text{ ng } \mu\text{l}^{-1}$) using either *osm-10:gfp* (A. Hart) or *rol-6dm* (C. Mello) as a marker. KP#286 and KP#287 were injected at $20 \text{ ng } \mu\text{l}^{-1}$ without marker, and lines were identified by the transgene GFP fluorescence. Three or more lines were analysed in all transgenic experiments.

Analysis of fluorescence

GLR-1::GFP was observed in living animals as described⁸. For confocal microscopy, animals were fixed in 4% paraformaldehyde, then observed on a BioRad MRC1045. All animals were confocally sectioned in successive $0.5 \mu\text{m}$ focal planes under identical conditions to allow comparison with NIH Image. Image data filled the 8-bit dynamic range without saturation.

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- Hebb, D. *The Organization of Behavior* (Wiley, New York, 1949).
- Stevens, C., Tonegawa, S. & Wang, Y. The role of calcium-calmodulin kinase II in three forms of synaptic plasticity. *Curr. Biol.* **4**, 687–693 (1994).
- Mayford, M., Wang, J., Kandel, E. & O'Dell, T. CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. *Cell* **81**, 891–904 (1995).
- Lisman, J., Malenka, R. C., Nicoll, R. A. & Malinow, R. Learning mechanisms: the case for CaM-KII. *Science* **276**, 2001–2002 (1997).
- Wu, G., Malinow, R. & Cline, H. Maturation of a central glutamatergic synapse. *Science* **274**, 972–976 (1996).
- Hart, A., Sims, S. & Kaplan, J. A synaptic code for sensory modalities revealed by analysis of the *C. elegans* GLR-1 glutamate receptor. *Nature* **378**, 82–85 (1995).
- Maricq, A. V., Peckol, E., Driscoll, M. & Bargmann, C. *glr-1*, a *C. elegans* glutamate receptor that mediates mechanosensory signalling. *Nature* **278**, 78–81 (1995).
- Rongo, C., Whitfield, C. W., Rodal, A., Kim, S. K. & Kaplan, J. M. LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. *Cell* **94**, 751–759 (1998).
- White, J. G., Southgate, E., Thomson, J. N. & Brenner, S. The structure of the nervous system of *Caenorhabditis elegans*. *Phil. Trans. R. Soc. Lond.* **314**, 1–340 (1986).
- Sulston, J. E. & Horvitz, H. R. Abnormal cell lineages in mutants of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **82**, 41–55 (1981).
- Turrigiano, G. G. Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci.* **22**, 221–227 (1999).
- Riddle, D., Blumenthal, T., Meyer, B. & Priess, J. (eds) *C. elegans II* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1997).
- Rao, A. & Craig, A. M. Activity regulates the synaptic localization of the NMDA receptor in hippocampal neurons. *Neuron* **19**, 801–812 (1997).
- O'Brien, R. J. et al. Activity-dependent modulation of synaptic AMPA receptor accumulation. *Neuron* **21**, 1067–1078 (1998).

15. Reiner, D. J., Newton, E. M., Tian, H. & Thomas, J. H. Diverse behavioural defects caused by mutations in *Caenorhabditis elegans unc-43* CaM Kinase II. *Nature* **402**, 199–203 (1999).
 16. Fong, Y. L., Taylor, W. L., Means, A. R. & Soderling, T. R. Studies of the regulatory mechanism of Ca²⁺/calmodulin-dependent protein kinase II. Mutation of threonine 286 to alanine and aspartate. *J. Biol. Chem.* **264**, 16759–16763 (1989).
 17. Schafer, W. & Kenyon, C. A calcium-channel homologue required for adaptation to dopamine and serotonin in *C. elegans*. *Nature* **375**, 73–78 (1995).
 18. Lee, R., Lobel, L., Hengartner, M., Horvitz, H. & Avery, L. Mutations in the alpha 1 subunit of an L-type voltage-activated Ca²⁺ channel cause myotonia in *Caenorhabditis elegans*. *EMBO J.* **16**, 6066–6076 (1997).
 19. Wu, G. Y. & Cline, H. T. Stabilization of dendritic arbor structure *in vivo* by CaMKII. *Science* **279**, 222–226 (1998).
 20. Nonet, M., Saifee, O., Zhao, H., Rand, J. & Wei, L. Synaptic transmission deficits in *Caenorhabditis elegans* synaptobrevin mutants. *J. Neurosci.* **18**, 70–80 (1998).
 21. Carroll, R. C., Lissin, D. V., von Zastrow, M., Nicoll, R. A. & Malenka, R. C. Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. *Nature Neurosci.* **2**, 454–460 (1999).
 22. Liao, D., Zhang, X., O'Brien, R., Ehlers, M. D. & Huganir, R. L. Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nature Neurosci.* **2**, 37–43 (1999).
 23. Shi, S. H. et al. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* **284**, 1811–1816 (1999).
 24. Shen, K. & Meyer, T. Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. *Science* **284**, 162–166 (1999).
 25. Barria, A., Derkach, V. & Soderling, T. Identification of the Ca²⁺/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate-type glutamate receptor. *J. Biol. Chem.* **272**, 32727–32730 (1997).
 26. Mammen, A. L., Kameyama, K., Roche, K. W. & Huganir, R. L. Phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II. *J. Biol. Chem.* **272**, 32528–32533 (1997).
 27. Kim, J. H. & Huganir, R. L. Organization and regulation of proteins at synapses. *Curr. Opin. Cell Biol.* **11**, 248–254 (1999).

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Diverse behavioural defects caused by mutations in *Caenorhabditis elegans unc-43* CaM Kinase II

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Calcium/calmodulin-dependent serine/threonine kinase type II (CaMKII) is one of the most abundant proteins in the mammalian brain, where it is thought to regulate synaptic plasticity and other processes^{1–3}. Activation of the multisubunit kinase⁴ by calcium is effectively cooperative and can persist long after transient calcium rises^{1,5,6}. Despite extensive biochemical characterization of CaMKII and identification of numerous *in vitro* kinase targets¹, little is known about its function *in vivo*. Here we report that *unc-43* encodes the only *Caenorhabditis elegans* CaMKII. A gain-of-function *unc-43* mutation reduces locomotory activity, alters excitation of three muscle types and lengthens the period of the motor output of a behavioural clock. Null *unc-43* mutations cause phenotypes generally opposite to those of the gain-of-function mutation. Mutations in the *unc-103* potassium channel gene suppress a gain-of-function phenotype of *unc-43* in one tissue

without affecting other tissues; thus, UNC-103 may be a tissue-specific target of CaMKII *in vivo*.

The kinase activity of each subunit of the multisubunit CaMKII holoenzyme is activated by binding Ca²⁺-calmodulin. Activated subunits can phosphorylate adjacent activated subunits, causing a large increase in affinity for Ca²⁺-calmodulin and partial Ca²⁺ independence, properties that confer cooperativity and memory of Ca²⁺ spikes^{5,6}. CaMKII can phosphorylate a wide range of proteins *in vitro*, but under these conditions the kinase is nonspecific¹ and the functions of these potential targets *in vivo* are unclear. *In vivo* evidence for function has come from deletion of the CaMKII α gene in mouse, which compromises spatial learning in the hippocampus and reduces its synaptic correlate, long-term potentiation^{2,3}. Further progress has been impeded by the extreme complexity of the mammalian brain and the existence of four CaMKII genes in mammals with overlapping expression patterns⁷, making genetic redundancy likely.

unc-43 is defined by one gain-of-function (*gf*) mutation and many loss-of-function (*lf*) mutations^{8,9}. We genetically mapped *unc-43* to an interval that contains the single *C. elegans* CaMKII gene (Fig. 1). We sequenced the CaMKII coding region¹⁰ in twelve *unc-43* alleles and found mutations in each (Fig. 1c). Two of the *lf*

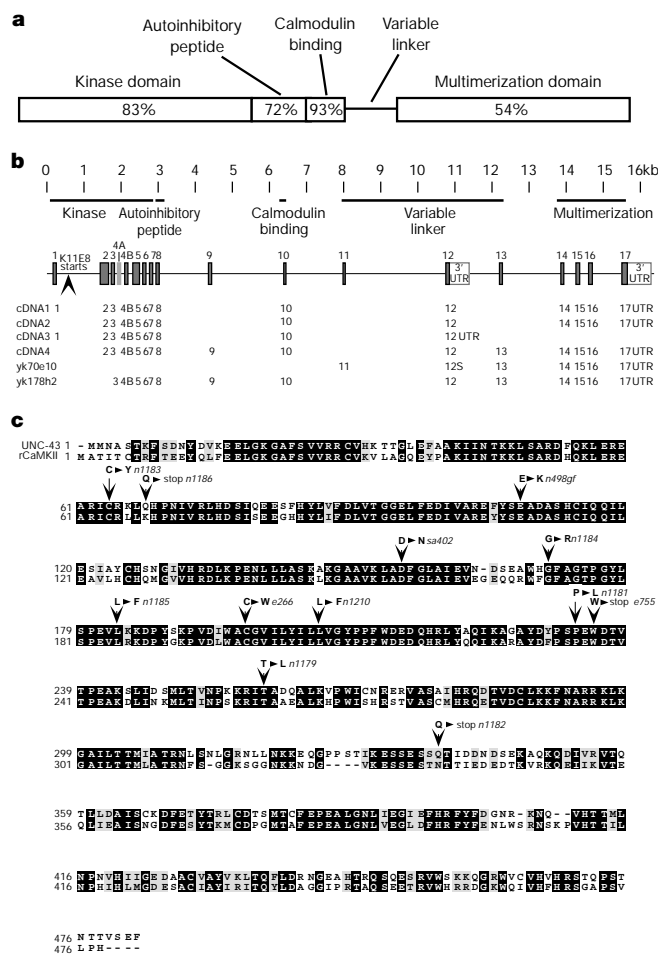


Figure 1 *unc-43* encodes CaMKII. **a**, cDNA1 has 69% overall identity with rat CaMKII α . *unc-43* functional domains are shown in boxes with their percentage identity. **b**, *unc-43* alternate splicing. The yk70e10 exon 12, '12S', is six nucleotides shorter than exon 12 in other cDNAs. Exon 4A was not identified in a cDNA, but might encode a peptide very similar to the exon 4B peptide. A similar arrangement of alternative exons 4 is found in the *Caenorhabditis briggsae* CaMKII homologue (not shown)¹⁰. All *unc-43* exons, but not introns, share a high degree of identity with the *C. briggsae* homologue (not shown). **c**, Alignment of *C. elegans unc-43* cDNA1 with rat CaMKII α . Black highlights identical residues, and grey highlights similar residues. Mutational changes are indicated.

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