Current Biology, Vol. 14, 2057–2062, November 23, 2004, ©2004 Elsevier Ltd. All rights reserved. DOI 10.1016/j.cub.2004.11.010

The Anaphase-Promoting Complex Regulates the Abundance of GLR-1 Glutamate Receptors in the Ventral Nerve Cord of *C. elegans*

Peter Juo[1] and Joshua M. Kaplan[1,*]
[1]Department of Molecular Biology
Massachusetts General Hospital
Department of Genetics
Harvard Medical School
Boston, MA 02114

Summary

The anaphase-promoting complex (APC) is a multisubunit E3 ubiquitin ligase that targets key cell cycle regulatory proteins for degradation. Blockade of APC activity causes mitotic arrest [1, 2]. Recent evidence suggests that the APC may have roles outside the cell cycle [3–6]. Several studies indicate that ubiquitin plays an important role in regulating synaptic strength [7–13]. We previously showed that ubiquitin is directly conjugated to GLR-1, a *C. elegans* non-NMDA (*N*-methyl-D-aspartate) class glutamate receptor (GluR), resulting in its removal from synapses [13]. By contrast, endocytosis of rodent AMPA GluRs is apparently regulated by ubiquitination of associated scaffolding proteins [12, 14]. Relatively little is known about the E3 ligases that mediate these effects. We examined the effects of perturbing APC function on postmitotic neurons in the nematode *C. elegans*. Temperature-sensitive mutations in APC subunits increased the abundance of GLR-1 in the ventral nerve cord. Mutations that block clathrin-mediated endocytosis blocked the effects of the APC mutations, suggesting that the APC regulates some aspect of GLR-1 recycling. Overexpression of ubiquitin decreased the density of GLR-1-containing synapses, and APC mutations blunted this effect. APC mutants had locomotion defects consistent with increased synaptic strength. This study defines a novel function for the APC in postmitotic neurons.

Results and Discussion

To investigate potential functions of the APC in neurons, we examined *C. elegans* mutants carrying mutations in APC subunits for changes in GLR-1 containing synapses. To visualize these synapses, we analyzed the distribution of GFP-tagged GLR-1 receptors (GLR-1::GFP). Expression of GLR-1::GFP in ventral-cord interneurons rescues the behavioral defects caused by *glr-1* null mutations, and the tagged receptors are localized in punctate structures in the ventral nerve cord [15, 16]. More than 80% of the GLR-1::GFP puncta are closely apposed to presynaptic markers (synaptobrevin and a vesicular glutamate transporter), suggesting that a large fraction of these puncta correspond to postsynaptic elements [13]. Five *C. elegans* APC subunits (*mat-1* CDC27, *emb-27* CDC16, *mat-2* APC1, *mat-3* CDC23, and *emb-30* APC4) exist as temperature-sensitive mutations that cause meiotic and mitotic defects [17–20]. To avoid mitotic defects, we shifted mutants to the restrictive temperature (25°C) during the fourth larval stage, after all somatic cell divisions have been completed [21]. We confirmed that this temperature shift regimen did not alter the number of cells expressing GLR-1::GFP (data not shown), suggesting that the pattern of cell divisions that generated these neurons had not been altered in the APC mutants. Therefore, we used this temperature shift protocol to look for postmitotic defects in the distribution of GLR-1::GFP.

We found that 20 hr after the shift to the restrictive temperature, the abundance of GLR-1::GFP in the ventral cord had increased in *mat-1* CDC27, *emb-27* CDC16, *mat-2* APC1, *mat-3* CDC23, and *emb-30* APC4 mutants compared to wild-type controls (Figure 1). To further document this effect, we analyzed the distribution of GLR-1::GFP in two APC mutants by using quantitative fluorescence microscopy. The amplitudes (%ΔF/F), widths, and density of GLR-1::GFP puncta in the ventral cord were measured as previously described [13]. The distribution of GLR-1::GFP in wild-type animals was temperature dependent, with puncta widths being significantly wider after the shift to 25°C (1.24 $\pm$ 0.06 μm at 20°C versus 1.55 $\pm$ 0.06 μm at 25°C). Despite this intrinsic temperature dependence, we found that GLR-1::GFP puncta widths were significantly increased in both *mat-1* CDC27 (18% increase, $p < 0.01$ t test) and *emb-27* CDC16 (26% increase, $p < 0.001$) mutants compared to wild-type controls subjected to identical temperature shift paradigms (Figures 1I–1K). By contrast, puncta amplitudes and densities did not change significantly in these mutants (Figures 1L–1M). In both cases, expression of wild-type *mat-1* CDC27 or *emb-27* CDC16 cDNAs rescued the defect in puncta widths observed in these mutants (Figures 1A–1E and 1I–1K). Expression of the *glr-1* promoter, which was used to drive expression of the APC cDNAs, begins in 3-fold embryos [22, 23], after the birth of the ventral-cord interneurons [24]. These results suggest that the APC functions cell autonomously in postmitotic ventral-cord interneurons to regulate the abundance or localization of GLR-1::GFP.

One potential explanation for our results is that the APC regulates the trafficking of membrane proteins generally or of synaptic proteins in particular. We examined the distribution of two other synaptic proteins in the ventral-cord interneurons: GFP-tagged synaptobrevin (*nuIs125*) (SNB-1::GFP, Figures 2A–2F) and LIN-10/Mint1 (LIN-10::GFP, Figures 2G–2K). SNB-1::GFP is localized to presynaptic nerve terminals [13, 15, 25]. LIN-10::GFP colocalizes with GLR-1 in the ventral nerve cord [15]. We found that the puncta amplitudes, widths, and densities of SNB-1::GFP (Figures 2D–2F) and LIN-10::GFP (Figures 2I–2K) were not significantly altered in APC mutants. Thus, the effects of the APC on GLR-1::GFP are relatively specific.

Substrate recognition by the APC is mediated by either of two activating subunits, CDH1 and CDC20. The amino termini of the APC activators CDH1 and CDC20

*Correspondence: kaplan@molbio.mgh.harvard.edu

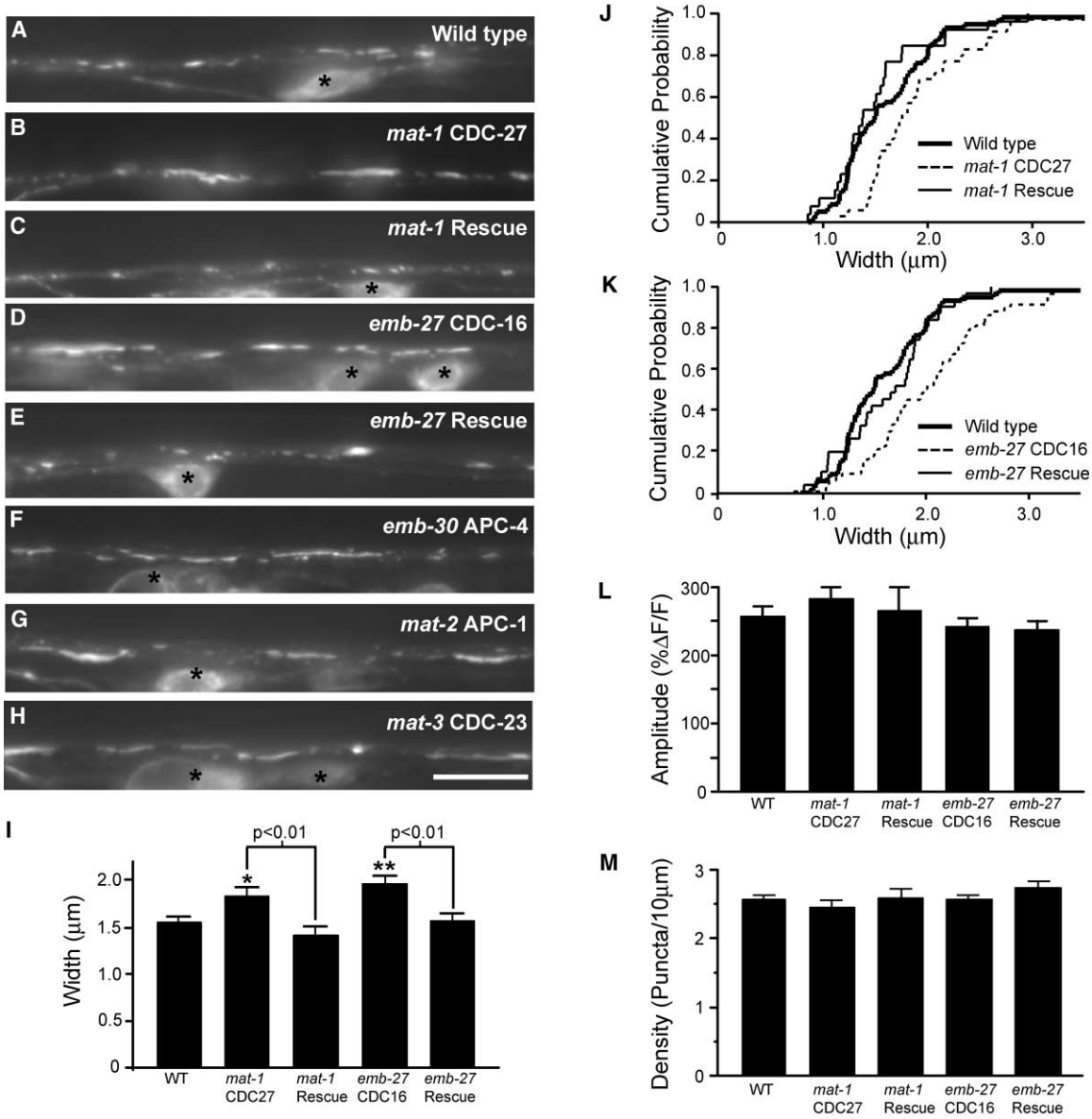

Figure 1. APC Subunits Function in Postmitotic Neurons to Regulate the Abundance of GLR-1::GFP

Stage four larvae (L4) of wild-type and APC mutants carrying the GLR-1::GFP transgene (*nuIs24*) were shifted from 15°C to 25°C. GLR-1::GFP puncta in the anterior ventral nerve cord of wild-type (A), *mat-1* CDC27 (B), *emb-27* CDC16 (D), *emb-30* APC-4 (F), *mat-2* APC-1 (G), and *mat-3* CDC23 (H) adult animals were imaged 20 hr later. The increased GLR-1::GFP puncta widths in *mat-1* CDC27 and *emb-27* CDC16 mutants was rescued by introduction of wild-type *mat-1* CDC27 (C) and *emb-27* CDC16 (E) cDNAs under the control of the *glr-1* promoter. Puncta widths (I), amplitudes (L), and densities (M), were compared in wild-type (WT; n = 60), *mat-1* CDC27 (n = 36), rescued *mat-1* CDC27 (n = 27), *emb-27* CDC16 (n = 59), and rescued *emb-27* CDC16 (n = 32) animals. (J and K) Cumulative probability histrograms of the puncta width data in (I) are shown. For this and all subsequent figures, anterior is to the left and ventral is up, asterisks indicate cell bodies of GLR-1-expressing cells, scale bars are 10 μm, error bars represent SEM, and values that differ significantly (Student's t test) from wild-type controls are indicated as follows: $p < 0.01$ (*) or $p < 0.001$ (**).

bind directly to substrates, and constructs driving the expression of the isolated substrate binding domains can function as dominant negatives [26]. Transgenic animals overexpressing DN-CDH1 (*nuIs133*) had significantly increased GLR-1::GFP puncta amplitudes and widths (Figure S1 in the Supplemental Data available with this article online), whereas those expressing DN-CDC20 had no effect (data not shown). These results are consistent with the idea that the CDH1-activated form of the APC regulates GLR-1 receptors; however, we cannot exclude the possibility that CDC20 is also involved. These results are consistent with reports indicating that CDH1 subunits are expressed in chick and rodent brains, whereas CDC20 subunits are not [4, 6].

We previously showed that overexpression of Myc-tagged ubiquitin (MUb) in the ventral-cord interneurons

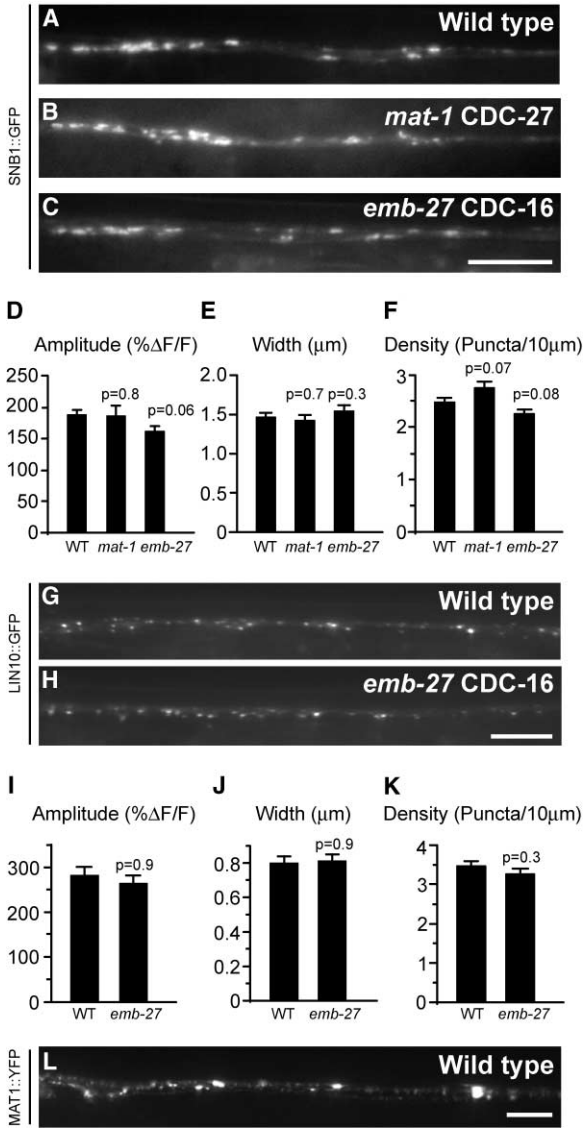

Figure 2. APC Mutants Did Not Affect the Distribution of GFP-Tagged Synaptobrevin and LIN-10/Mint1

L4 stage wild-type and temperature-sensitive larvae carrying the synaptobrevin::GFP (SNB-1::GFP) transgene under the control of the *glr-1* promoter (*nuIs125*) were shifted from 15°C to 25°C. SNB-1::GFP puncta in the anterior ventral nerve cord of wild-type (A), *mat-1* CDC27 (B), and *emb-27* CDC16 (C) adult animals were imaged 20 hr later. Synaptobrevin puncta amplitudes (D), widths (E), and densities (F) were compared in wild-type (n = 32), *mat-1* CDC27 (n = 18), and *emb-27* CDC16 (n = 29) animals. The scale bar (10 μm) shown in panel (C) applies to panels (A–C). Wild-type and temperature-sensitive L4 larvae carrying the LIN-10::GFP transgene under the control of the *glr-1* promoter (*nuEx*993) were shifted from 15°C to 25°C. LIN-10::GFP puncta in the anterior ventral nerve cord of wild-type (G) and *emb-27* CDC16 (H) adult animals were imaged 20 hr later. LIN-10::GFP puncta amplitudes (I), widths (J), and densities (K) were compared between wild-type (n = 32) and *emb-27* CDC16 (n = 29) animals. The scale bar (10 μm) shown in (H) applies to (G) and (H). (L) The distribution of YFP-tagged MAT-1/CDC27 (MAT1::YFP) in the ventral cord after expression with the *glr-1* promoter is shown.

results in the formation of MUb-GLR-1 conjugates and decreases the amplitude and density of GLR-1::GFP puncta (Figure 3) [13]. We found that *mat-1* CDC27 (Figures 3C and 3F) and *emb-27* CDC16 (Figures 3D and 3F) mutations both significantly decreased the effects of MUb overexpression on the density of GLR-1::GFP puncta. The *mat-1* CDC27 mutation also decreased the effect of MUb on puncta amplitudes, whereas the *emb-27* CDC16 mutation did not (Figure 3E). These results suggest that the APC is one of the E3 ligases that mediates the effects of ubiquitin on GLR-1-containing synapses.

One potential explanation for our results is that GLR-1 is a direct target of the APC. Several results suggest that this is unlikely. First, GLR-1 does not contain any predicted D-box or KEN-box motifs, which mediate recognition of APC targets [1, 2]. Second, *mat-1* CDC27 and *emb-27* CDC16 mutations partially blocked the ubiquitin-induced decrease in GLR-1::GFP puncta density (Figure 3F). In contrast, a GLR-1 mutant that lacks cytoplasmic lysine residues (GLR-1[4KR]) and is not ubiquitinated did not block the ubiquitin-induced decrease in puncta density [13]. Third, if changes in ubiquitination of GLR-1 are required for APC-mediated regulation of GLR-1::GFP, then the GLR-1(4KR) mutation should occlude the effects of *emb-27* CDC16 mutations on GLR-1 puncta. Instead, we found that puncta widths in GLR-1 (4KR) single mutants (1.72 ± 0.08 μm, n = 25 animals) were significantly smaller than those observed in GLR-1 (4KR);*emb-27* CDC16 double mutants (2.0 ± 0.08 μm, n = 34 animals, p = 0.027). Fourth, the abundance of Ub-GLR-1 conjugates in *emb-27* CDC16 mutants (0.25 ± 0.03% total GLR-1, n = 12 experiments) was not significantly different from that observed in wild-type extracts (0.2 ± 0.02%, n = 8 experiments) (Figures 3G–3H). Fifth, GLR-2, a second GluR subunit that is likely to form hetero-oligomeric GluRs with GLR-1 [27] has a KEN-box sequence; however, mutating this sequence in GLR-2 had no obvious effect on the distribution of GLR-1::GFP and did not prevent the ubiquitin-induced decreases in GLR-1::GFP (Figure S2). These results suggest that the effect of the APC on GLR-1::GFP puncta cannot be explained by a change in the ubiquitination of either GLR-1 or GLR-2.

The increased abundance of GLR-1::GFP observed in APC mutants could be caused by increased transcription from the *glr-1* promoter. We addressed this possibility by analyzing expression of a *glr-1* transcriptional reporter construct, *nuIs1* (*Pglr1::GFP*), and with real time PCR. We found that the expression of *nuIs1* was not significantly different in the ventral nerve cords of wild-type, *mat-1* CDC27, and *emb-27* CDC16 animals (average intensity values ± SEM: wild-type: 1673 ± 51 AU, n = 29; *mat-1* CDC27: 1614 ± 50 AU, n = 23; *emb-27* CDC16: 1585 ± 70 AU, n = 17). Real-time PCR experiments found that the abundance of GLR-1::GFP mRNA in *emb-27* CDC16 and *mat-1* CDC27 mutants was approximately 44% and 61%, respectively, of that in wild-type controls. These results suggest that the increased abundance of GLR-1::GFP in the ventral cord of APC mutants was not caused by increased expression of GLR-1.

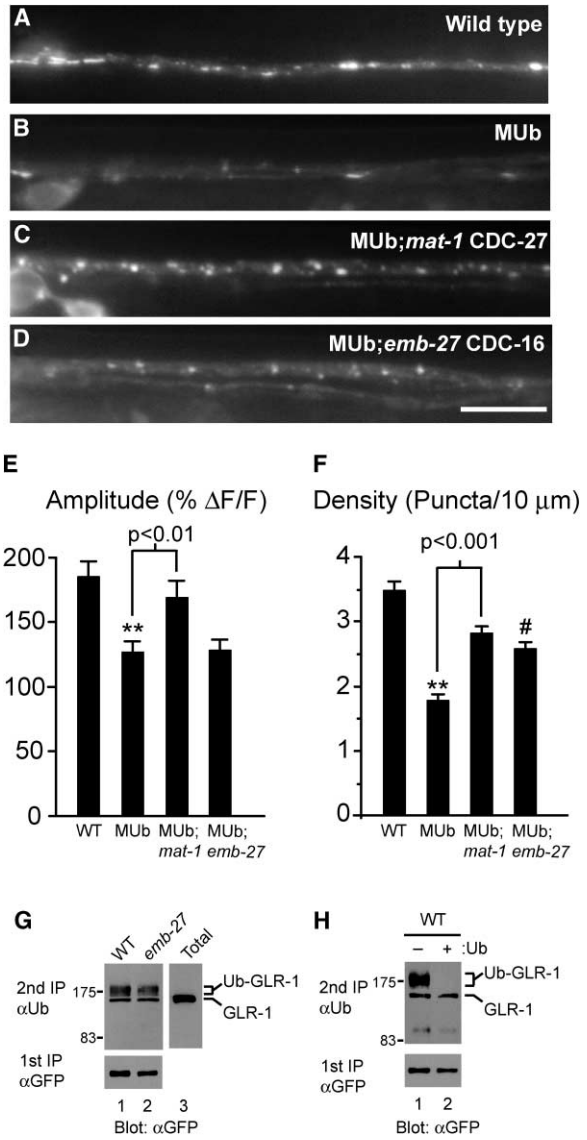

Figure 3. APC Mutations Prevented the Ubiquitin-Induced Decrease in GLR-1::GFP Puncta Density

GLR-1::GFP puncta (*nuls25*) in the anterior ventral nerve cords of wild-type (A), *nuls89* MUb (B), and double mutants *nuls89* MUb;*mat-1* CDC27 (C) and *nuls89* MUb;*emb-27* CDC16 (D) were imaged. Puncta amplitudes (E), and densities (F) were compared in wild-type ($n = 34$), *nuls89* MUb ($n = 38$), *nuls89* MUb;*mat-1* CDC27 ($n = 27$), and *nuls89* MUb;*emb-27* CDC16 MUb ($n = 28$) animals. MUb;*emb-27* CDC-16 puncta density was statistically different from that with MUb alone: $p < 0.001$ (#). For all experiments, L4 larvae were shifted from 15°C to 25°C and imaged as adults 20 hr later. (G) Solubilized membrane fractions from wild-type (lane 1) or *emb-27* CDC16 (lane 2) mutant worms were subjected to a double immunoprecipitation protocol [13]. Extracts were first immunoprecipitated (IP) with anti-GFP (1st IP) to pull down GLR-1::GFP, then immunoprecipitated with anti-ubiquitin (2nd IP) and subsequently immunoblotted with anti-GFP. Unmodified GLR-1::GFP was detected in total membrane proteins from wild-type animals immunoblotted with anti-GFP (lane 3). In lanes 1 and 2, 180-fold more extract was used than was loaded in lane 3. The bottom panel shows that roughly equal amounts of total GLR-1::GFP were loaded from mutant and wild-type extracts. (H) Solubilized membrane fractions from wild-type worms were subjected to the same double immunoprecipitation protocol described above, except that in the second IP the ubiquitin antibody was preblocked either with BSA (lane 1) or with excess free ubiquitin (lane 2). The positions of unmodified GLR-1::GFP (GLR-1) and ubiquitinated GLR-1 (Ub-GLR-1) are indicated on the right. Molecular-weight markers are indicated on the left (kDa). WT refers to *nuls24* GLR-1::GFP, and *emb-27* refers to *nuls24* GLR-1::GFP;*emb-27*. For experiments shown in (G) and (H), L4 larvae were shifted from 15°C to 25°C, and extracts were prepared 20 hr later.

Synaptic GLR-1 receptors are likely to be derived from a pool of recycling receptors undergoing exocytosis, endocytosis, and postendocytic degradation, as has been shown for mammalian AMPA receptors [28–31]. Indeed, the synaptic abundance of GLR-1::GFP is regulated by ubiquitin and clathrin-mediated endocytosis [13]. If the accumulation of GLR-1::GFP at synapses in APC mutants was caused by a change in recycling of GLR-1, then mutations that block endocytosis should prevent membrane recycling and thereby block the effects of the APC on GLR-1. To disrupt endocytosis, we used strains containing an *unc-11* mutation. The *unc-11* gene encodes AP180, a clathrin adaptin protein that is required for endocytosis [13, 32–34]. We found that puncta widths in *unc-11* AP180;*emb-27* CDC16 double mutants (1.91 ± 0.12 μm, $n = 27$ animals) were not significantly different from those in *unc-11* AP180 single mutants (2.17 ± 0.15 μm, $n = 22$ animals, $p = 0.46$, Kolmogorov-Smirnov [KS] test), as would be predicted if the increased puncta widths in APC mutants were caused by a change in the recycling of GLR-1 (Figure S3).

If the APC regulates some aspect of recycling of GLR-1 in dendrites, then we would expect that APC subunits should be present in the ventral nerve cord. We visualized the intracellular distribution of MAT-1/CDC27 by expressing a YFP-tagged MAT-1 construct in the ventral-cord interneurons. We found that YFP-MAT-1/CDC27 was localized in prominent punctate structures in the ventral nerve cord (Figure 2L). The YFP-MAT-1/CDC27 puncta were typically not well correlated with the positions of GLR-1::CFP puncta (data not shown). These results are consistent with the idea that the APC acts in the ventral cord to regulate local recycling of GLR-1 receptors.

If APC mutants have increased levels of GLR-1 receptors on the cell surface, we would expect synaptic signaling would be altered, which should lead to a change in behavior. Spontaneous *C. elegans* locomotion consists of an alternating pattern of forward and reverse sinusoidal movements. The GLR-1-containing synapses regulate the duration of forward locomotion (Figure 4A). Mutants with decreased glutamatergic transmission, e.g., those lacking GLR-1 or a vesicular glutamate transporter, have a pattern of spontaneous locomotion characterized by prolonged periods of forward movement [35]. By contrast, mutants that have increased glutamatergic transmission have shorter periods of forward movement [13, 27] (Figure 4A). We found that *mat-1* CDC27 and *emb-30* APC4 mutants had decreased durations of forward movement (Figures 4B and 4C) (*ax144ts* $p < 0.001$, *ax212ts* $p < 0.001$, and *emb-30* $p < 0.05$; KS test). This change in locomotion was observed in strains containing two independent alleles of both *mat-1* CDC27 and *emb-30* APC4 (Figures 4B and 4C). If the change in locomotion was caused by increased signal-

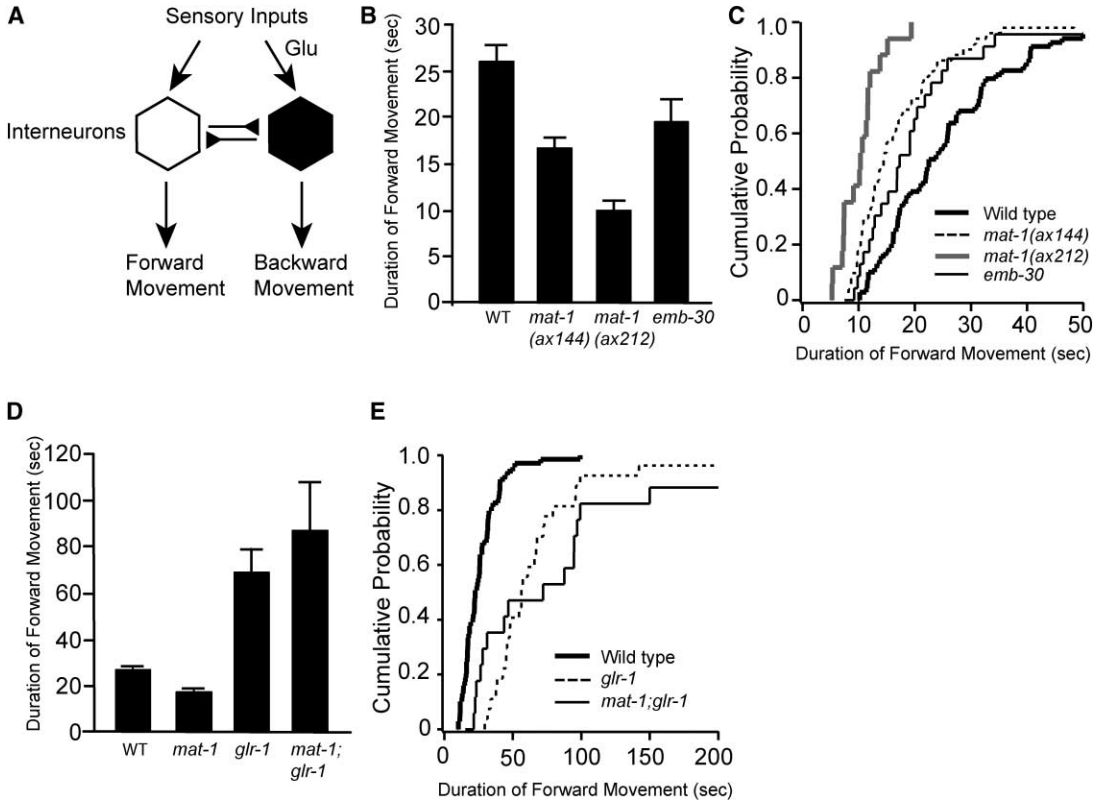

Figure 4. APC Mutations Alter Locomotion Behavior in a *glr-1*-Dependent Manner

(A) A simplified model of the *C. elegans* neural circuit for locomotion. GLR-1-expressing interneurons drive either forward (AVB and PVC, white hexagons) or backward (AVA and AVD, black hexagons) movement. The forward-and backward-driving interneurons form extensive connections with each other [37, 38]. Glutamatergic input from sensory neurons bias the circuit toward backward movement [35].

(B) The average duration of forward locomotion was compared in wild-type (WT; n = 70), *mat-1(ax144ts)* CDC27 (n = 52), *mat-1(ax212ts)* CDC27 (n = 18), and *emb-30* APC4 mutant animals. The *emb-30* APC4 data represent pooled results for two alleles, *g53ts* (n = 12) and *tn377ts* (n = 12).

(C) The data from (A) are plotted as a cumulative probability histogram. In all cases, the behavior of APC mutants was significantly different (Kolmogorov-Smirnov [KS] test) from that observed in wild-type controls: *ax144ts* $p < 0.001$, *ax212ts* $p < 0.001$, and *emb-30* $p < 0.05$.

(D) The average duration of forward locomotion was compared in wild-type (n = 70), *mat-1(ax144ts)* CDC27 (n = 52), *glr-1(n2461)* (n = 28), and *mat-1(ax144ts);glr-1(n2461)* (n = 18) double mutants.

(E) Cumulative probability histograms of the data in (D) are shown. The behavior of *glr-1* single mutants and *mat-1* CDC27;*glr-1* GluR double mutants was not significantly different ($p = 0.18$; KS test). L4 larvae were shifted from 15°C to 25°C, and adult animals were assayed for locomotion behavior 20 hr later.

ing at GLR-1-containing synapses, then this effect should depend upon the expression of endogenous GLR-1 receptors. We found that the average duration of forward locomotion in *glr-1* single mutants was indistinguishable from that observed in *mat-1* CDC27; *glr-1* GluR double mutants (Figures 4D and 4E) ($p = 0.18$; KS test). These results suggest that the APC alters locomotion via regulation of endogenously expressed GLR-1 receptors. Therefore, decreased APC activity was associated with increased GLR-1::GFP fluorescence in ventral cord puncta and with locomotion defects consistent with increased synaptic strength.

We previously reported that ubiquitin promotes loss of GLR-1-containing synapses and produces behavioral changes consistent with reduced synaptic strength [13]. Our current study identifies the APC as one of the E3 enzymes involved in this process. Our results indicate that the APC functions in postmitotic neurons to regulate the abundance of GLR-1 receptors at postsynaptic elements. Interestingly, the APC regulates multiple aspects of postmitotic neurons; such aspects include GluR trafficking and axon outgrowth [5]. The APC target mediating axon outgrowth has not been identified. Neither GLR-1 nor GLR-2 is likely to be the APC substrate mediating the effects on GluRs. Mammalian GluRs have been shown to bind a number of scaffolding proteins [28–31]. Several of these scaffolding proteins undergo activity-dependent ubiquitination [12, 36]. Thus, it is possible that APC regulation is mediated by ubiquitination of a scaffolding protein associated with GLR-1. Many postsynaptic proteins undergo concerted changes in ubiquitination in response to changes in synaptic activity [36]. Consequently, it is likely that the activity of E3 ligases will be regulated and that this may provide a mechanism underlying some forms of activity-dependent plasticity.

Supplemental Data

Supplemental Experimental Procedures and three additional figures are available with this article online at http://www.current-biology.com/cgi/content/full/14/22/2057/DC1/.

Acknowledgments

We thank the following for strains, reagents, and advice: M. Wellenfang, G. Seydoux, A. Golden, D. Shakes, J. Schumacher, N. Hawkins, G. Garriga, B. Barstead, V. Maricq, L. Dreier, J. Dittman, M. Burbea, J. McEwen, M. Grunwald, T. Alioto, C. Janson, and the *C. elegans* Genetics Stock Center. We thank A. Brand for sharing results prior to publication. We thank members of the Kaplan lab for critical comments on this manuscript. This work was supported by a grant from the National Institutes of Health (NIH) to J.M.K. (NS32196). P.J. was supported by postdoctoral fellowships from the NIH (1F32NS10957) and the American Cancer Society (PF-00-149-01-CSM).

Received: January 16, 2004
Revised: September 27, 2004
Accepted: September 29, 2004
Published: November 23, 2004

References

1. Harper, J.W., Burton, J.L., and Solomon, M.J. (2002). The anaphase-promoting complex: it's not just for mitosis any more. Genes Dev. *16*, 2179–2206.
2. Peters, J.M. (2002). The anaphase-promoting complex: proteolysis in mitosis and beyond. Mol. Cell *9*, 931–943.
3. Wirth, K.G., Ricci, R., Gimenez-Abian, J.F., Taghybeeglu, S., Kudo, N.R., Jochum, W., Vasseur-Cognet, M., and Nasmyth, K. (2004). Loss of the anaphase-promoting complex in quiescent cells causes unscheduled hepatocyte proliferation. Genes Dev. *18*, 88–98.
4. Wan, Y., and Kirschner, M.W. (2001). Identification of multiple CDH1 homologues in vertebrates conferring different substrate specificities. Proc. Natl. Acad. Sci. USA *98*, 13066–13071.
5. Konishi, Y., Stegmuller, J., Matsuda, T., Bonni, S., and Bonni, A. (2004). Cdh1-APC controls axonal growth and patterning in the mammalian brain. Science *303*, 1026–1030.
6. Gieffers, C., Peters, B.H., Kramer, E.R., Dotti, C.G., and Peters, J.M. (1999). Expression of the CDH1-associated form of the anaphase-promoting complex in postmitotic neurons. Proc. Natl. Acad. Sci. USA *96*, 11317–11322.
7. Cline, H. (2003). Synaptic plasticity: Importance of proteasome-mediated protein turnover. Curr. Biol. *13*, R514–R516.
8. Hegde, A.N., and DiAntonio, A. (2002). Ubiquitin and the synapse. Nat. Rev. Neurosci. *3*, 854–861.
9. Murphey, R., and Godenschwege, T. (2002). New roles for ubiquitin in the assembly and function of neuronal circuits. Neuron *36*, 5.
10. Speese, S.D., Trotta, N., Rodesch, C.K., Aravamudan, B., and Broadie, K. (2003). The ubiquitin proteasome system acutely regulates presynaptic protein turnover and synaptic efficacy. Curr. Biol. *13*, 899–910.
11. Zhao, Y., Hegde, A.N., and Martin, K.C. (2003). The ubiquitin proteasome system functions as an inhibitory constraint on synaptic strengthening. Curr. Biol. *13*, 887–898.
12. Colledge, M., Snyder, E.M., Crozier, R.A., Soderling, J.A., Jin, Y., Langeberg, L.K., Lu, H., Bear, M.F., and Scott, J.D. (2003). Ubiquitination regulates PSD-95 degradation and AMPA receptor surface expression. Neuron *40*, 595–607.
13. Burbea, M., Dreier, L., Dittman, J.S., Grunwald, M.E., and Kaplan, J.M. (2002). Ubiquitin and AP180 regulate the abundance of GLR-1 glutamate receptors at postsynaptic elements in C. elegans. Neuron *35*, 107–120.
14. Patrick, G.N., Bingol, B., Weld, H., Reis, G., and Schuman, E. (2003). Ubiquitin-mediated proteasome activity is required for agonist-induced endocytosis of GluRs. Curr. Biol. *13*, 2073–2081.
15. Rongo, C., Whitfield, C.W., Rodal, A., Kim, S.K., and Kaplan, J.M. (1998). LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. Cell *94*, 751–759.
16. Rongo, C., and Kaplan, J. (1999). CaMKII regulates the density of central glutamatergic synapses in vivo. Nature *402*, 195–199.
17. Wallenfang, M.R., and Seydoux, G. (2000). Polarization of the anterior-posterior axis of C. elegans is a microtubule-directed process. Nature *408*, 89–92.
18. Golden, A., Sadler, P.L., Wallenfang, M.R., Schumacher, J.M., Hamill, D.R., Bates, G., Bowerman, B., Seydoux, G., and Shakes, D.C. (2000). Metaphase to anaphase (mat) transition-defective mutants in Caenorhabditis elegans. J. Cell Biol. *151*, 1469–1482.
19. Furuta, T., Tuck, S., Kirchner, J., Koch, B., Auty, R., Kitagawa, R., Rose, A.M., and Greenstein, D. (2000). EMB-30: an APC4 homologue required for metaphase-to-anaphase transitions during meiosis and mitosis in Caenorhabditis elegans. Mol. Biol. Cell *11*, 1401–1419.
20. Davis, E.S., Wille, L., Chestnut, B.A., Sadler, P.L., Shakes, D.C., and Golden, A. (2002). Multiple subunits of the Caenorhabditis elegans anaphase-promoting complex are required for chromosome segregation during meiosis I. Genetics *160*, 805–813.
21. Sulston, J.E., and Horvitz, H.R. (1977). Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. *56*, 110–156.
22. Hart, A., Sims, S., and Kaplan, J. (1995). A synaptic code for sensory modalities revealed by analysis of the *C. elegans* GLR-1 glutamate receptor. Nature *378*, 82–85.
23. Maricq, A.V., Peckol, E., Driscoll, M., and Bargmann, C. (1995). *glr-1*, a *C. elegans* glutamate receptor that mediates mechanosensory signaling. Nature *378*, 78–81.
24. Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *Caenorhabditis elegans*. Dev. Biol. *100*, 64–119.
25. Nonet, M.L. (1999). Visualization of synaptic specializations in live C. elegans with synaptic vesicle protein-GFP fusions. J. Neurosci. Methods *89*, 33–40.
26. Pfleger, C.M., Lee, E., and Kirschner, M.W. (2001). Substrate recognition by the Cdc20 and Cdh1 components of the anaphase-promoting complex. Genes Dev. *15*, 2396–2407.
27. Mellem, J.E., Brockie, P.J., Zheng, Y., Madsen, D.M., and Maricq, A.V. (2002). Decoding of polymodal sensory stimuli by postsynaptic glutamate receptors in C. elegans. Neuron *36*, 933–944.
28. Malinow, R., and Malenka, R.C. (2002). AMPA receptor trafficking and synaptic plasticity. Annu. Rev. Neurosci. *25*, 103–126.
29. McGee, A.W., and Bredt, D.S. (2003). Assembly and plasticity of the glutamatergic postsynaptic specialization. Curr. Opin. Neurobiol. *13*, 111–118.
30. Sheng, M., and Kim, M.J. (2002). Postsynaptic signaling and plasticity mechanisms. Science *298*, 776–780.
31. Song, I., and Huganir, R.L. (2002). Regulation of AMPA receptors during synaptic plasticity. Trends Neurosci. *25*, 578–588.
32. Kirchhausen, T. (1999). Adaptors for clathrin-mediated traffic. Annu. Rev. Cell Dev. Biol. *15*, 705–732.
33. Nonet, M.L., Holgado, A.M., Brewer, F., Serpe, C.J., Norbeck, B.A., Holleran, J., Wei, L., Hartwieg, E., Jorgensen, E.M., and Alfonso, A. (1999). UNC-11, a Caenorhabditis elegans AP180 Homologue, Regulates the Size and Protein Composition of Synaptic Vesicles. Mol. Biol. Cell *10*, 2343–2360.
34. Zhang, B., Koh, Y.H., Beckstead, R.B., Budnik, V., Ganetzky, B., and Bellen, H.J. (1998). Synaptic vesicle size and number are regulated by a clathrin adaptor protein required for endocytosis. Neuron *21*, 1465–1475.
35. Zheng, Y., Brockie, P.J., Mellem, J.E., Madsen, D.M., and Maricq, A.V. (1999). Neuronal control of locomotion in C. elegans is modified by a dominant mutation in the GLR-1 ionotropic glutamate receptor. Neuron *24*, 347–361.
36. Ehlers, M.D. (2003). Activity level controls postsynaptic composition and signaling via the ubiquitin-proteasome system. Nat. Neurosci. *6*, 231–242.
37. Chalfie, M., Sulston, J.E., White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1985). The neural circuit for touch sensitivity in *C. elegans*. J. Neurosci. *5*, 956–964.
38. White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of *Caenorhabditis elegans*. Philos. Trans. R. Soc. Lond. *314*, 1–340.

Note Added in Proof

Similar effects for the APC at the *Drosophila* neuromuscular junction were reported by van Roessel et al. (Independent regulation of synaptic size and activity by the anaphase-promoting complex (2004). Cell *119*, in press).