**The Retrotransposon Copia regulates structural synaptic plasticity at the Drosophila Neuromuscular Junction**

By

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**ABSTRACT**

Despite two decades of solid advances in sequencing genomes from an ever-increasing number of species, the function of a large part of most genomes, the so called “junk DNA”, remains largely unknown. Much of this enigmatic DNA corresponds to genomic parasites - transposons. However, the domesticated transposon fragment, *arc* (*activity-regulated cytoskeleton-associated protein*), has been recently shown to serve as a mechanism to transport RNAs across the synapse. Here we show the transposon Copia is enriched at the Drosophila neuromuscular junction, it is transported across synapses in extracellular vesicles and antagonizes Arc. Copia downregulation results in abnormal synapse development and increased synaptic plasticity. To our knowledge, this is the first report documenting a physiological role of a transposon at synapses, lending further weight to recent arguments and data suggesting transposons and potentially other types of “junk DNA” are not junk after all.

**Keywords**

Copia, Arc/Arg3.1, plasticity, retrotransposon, capsid, RNA trafficking, extracellular vesicles, transsynaptic RNA transport, RNA-binding protein.

**INTRODUCTION**

Mechanisms of communication across synapses are diverse, as exemplified by recent studies identifying a novel mechanism, the ViSyToR (Viral Synaptic Transfer of RNA) pathway, by which proteins and RNAs are transported across synaptic compartments to influence development of postsynaptic sites1,2. At the center-stage of this pathway is a domesticated transposable element (TE) fragment, *arc*. In Drosophila, Arc (dArc1) is in extracellular vesicles (EVs), forms a viral-like capsid, and transfers across synaptic boutons at larval neuromuscular junctions (NMJs) 2. Notably, this transfer is required for proper NMJ development and structural synaptic plasticity. The viral-like activity of Arc is also observed in mammals3.

Large portions of eukaryotic genomes (95% in humans; 50% in flies) are encoded by the so called “junk DNA”. A significant part of junk DNA in the human genome is comprised of TEs, with estimates ranging from 45-60% of the genome4. In humans, copies of one active retro-TE, Long interspersed nuclear element-1 (LINE-1), comprise ~20% of the genome 5. Some TEs, such as the retro-TE Ty3/Gypsy superfamily, possess similarities to viruses, with some being indistinguishable from retroviruses, such as HIV-1 in terms of conserved domains (Fig. 1A) 5. Analogous to retroviruses, Gypsy in Drosophila has a gag region encoding capsid proteins that encapsulate the viral genome; they also often have very similar polymerase (pol) sequences which are translated into enzymes needed for reverse transcription of the viral RNA genome and integration of the viral/TE DNA genome into the host genome; they also contain an envelope (env) region, encoding proteins needed for viral membrane maturation. In some instances, both TEs and viruses (e.g., the polio virus) lack the env region 6.

The “hopping” of TEs throughout a genome is thought to be parasitic. Evidence suggests that TE activation is coincident with, if not partially causal of, some diseases7. In addition, genomic TE insertions are widely regarded as major drivers of evolution 8,9. Admittedly, many genes have TE-like sequences that contribute to their expression and/or function. For example, placenta formation in mammals and the somatic recombination (V(D)J junction) needed for antibody production are both products of TE domestication10,11. The telomerase activity required for telomere expansion is adapted from and dependent upon TEs; some species’ telomeres are completely comprised of or controlled by TEs 12. Another example of TE domestication is the highly conserved gene *arc*, the TE/viral-like activity of which is needed to regulate synaptic plasticity 13.

In the Drosophila genome, one of the more abundant TEs is *copia*. Copia mRNA is a highly enriched, if not the most abundant, transcript in some strains of cultured Drosophila cells 14. After the initial discovery of TEs by McClintock 15, TEs failed to garner scientific interest until the molecular revolution of the 1970s. Initial sequencing experiments brought to light the viral-like properties of TEs. Copia served as a critical source of insight into these properties. In part, Copia was used to show that TEs have reverse transcriptase and form viral-like intermediates during replication 16-18. It is now clear that TEs and viruses are integrally linked and show striking similarities (Fig. 1).

Our observations on dArc1 prompted us to look for other molecules transported across presynaptic compartments in this viral-like manner, and we discovered Copia is enriched in EVs 2. This led to the current investigation into Copia, where we found the capsid region of *copia* is enriched at and transfers across Drosophila NMJs. We further observed Copia protein can auto-assemble into capsids. Knocking down Copia at the NMJ leads to a striking increase in structural synaptic plasticity. We observe Copia and dArc1 are in an antagonistic relationship phenotypically and genetically. Taken together, our observations suggest Copia is a new player in NMJ plasticity that may interact with a well-established plasticity pathway. This novel form of TE domestication provides some of the first *in vivo* evidence that TEs play a pivotal role in neuronal development.

**RESULTS**

The transposon Copia is in EVs.

We previously found a spliced form of Copia mRNA, Copiagag, is enriched in EVs derived from Drosophila Schneider 2 (S2) cultured cells 2. This seems to be S2 strain-specific, as previous work shows that it is full-length copia, not copiagag,that is enriched in EVs in Drosophila cultured adherent cells, S2R+, as compared to the S2 cells used in this work19. Copiagag has long been observed in S2 cells as being competent to form capsid-like structures20.

To test if Copia protein is present in Drosophila EVs we developed a series of tools, including an antibody generated against Copia (α-CopiaFull), which recognizes peptides encoded by the GAG and POL regions, and can detect the full-length unspliced Copia peptide (Fig. 1B-C). We further generated another antibody, α-Copiagag, against a peptide that is specifically encoded by the *copiagag* spliced transcript (Fig. 1B-C). To verify these antibodies, we tested them at the Drosophila NMJ with copia-specific RNAi constructs (see below) and, as well with α-Copiagag, we carried out peptide competition assays (Fig. S1H). Further with α-Copiagag as Copiagag is predicted to not encode the reverse transcriptase domain of Copia we tested α-Copiagag IP fractions for RT activity and found none (Fig. S1G). Using S2 lysates with α-Copiagag we detect a very prominent band at 50kDa, the predicted size of Copiagag (Fig. 1C). Likewise, using α-CopiaFull, we observe several discrete bands, some corresponding to the size of predicted or known synthetic full length Copia-derived peptides (Fig. 1C). The presence of peptides of varying sizes is consistent with viral peptide auto-cleaving, which is typically observed in viruses where the full-length peptides are cleaved into functional small peptides 20. This is in line with the viral nature of a TE such as Copia 21,22.

To assess whether the antibody was working as expected, i.e., it labelled EVs which are known to contain Copia, we conducted immunocytochemistry of EVs using α-Copiagag. Briefly, we isolated EVs from S2 cells, exposed them to saponin treatment to remove EV membranes, then incubated EVs with α-Copia (Fig. 1D and S2A)**.**

Copiagag forms capsids and associates with its own transcript

The spliced form of Copia, Copiagag, contains the complete capsid, encoded in the GAG region, and a small part of the POL region. As Copia is a TE that has been in Drosophila genomes for millions of years it is possible that Copia has lost its envelope found on TEs like Gypsy (Fig. 1A). We observe bacterially expressed Copiagag (Fig. S2B) can auto-assemble into capsid-like structures 23,24 (Fig. 2A). Synthetically expressed Copiagag does not form uniformly sized capsids, ranging in size from 40nm to 1700nmwith an average size of 81.2nm; the most abundant size being 65nm and with >90% falling between 40-120nm (Fig. 2B).

A traditional role of capsid proteins is to surround and protect the viral genome. Thus, we tested if Copiagag associates with *copia* transcript. We conducted RNA immunoprecipitations (RIP) of S2 cell extracts using α-Copiagag and α-CopiaFull. We then tested for the presence of *copiagag*and *copiafull* transcripts using digital qPCR (dPCR) and probes that distinguish *copiagag* from *copiafull* RNA isoforms. From CNS and BWM, we find that both α-Copiafull and α-Copiagag immunoprecipitate copia transcript (Fig. 2C and 2D). Taken together, these results show Copia antibodies associate with Copia transcripts, and that Copia antibodies recognize Copia peptides, thus supporting a model that Copia forms capsids in vivo, likely encapsulates its own transcript.

Using digital long RNA-seq (nanopore see materials and methods) we found that in the CNS Copiagag is enriched 1.64 compared to Copiafull transcript, while in the BWM Copiagag shows a ratio of 0.517 compared to Copiafull, which was confirmed by dPCR (Fig. 2E and S5B-C). The copia antibodies, did not co-IP with Rpl32 nor dArc1 (Fig. S2E-F). Western-blot of tissues using α-CopiaFull further reveals a 50kDA isoform, corresponding to the predicted Copiagag size and the predominant Copia species in larval CNS. Bands of several sizes are observed with the same antibody in protein extracts from larval body walls (Fig. 2F). Using α-Copiagag we found a predominant band migrating at the predicted molecular weight of Copiagag in larval CNS, suggesting Copiagag is enriched in larval CNS (Fig. 2G). As viruses are known to autocleave using their protease (PR) domain, which is present in both CopiaFull and Copiagag peptides, the multitude of bands observed in body walls could be potentially explained by autocleavage.

Copiagag is enriched at and transfers across the Drosophila NMJ.

Immunolabelling with α-Copiagag reveals distinct localization patterns in the larval CNS and other tissues (Fig. S3 A and B), with a striking enrichment at the Drosophila NMJ (Fig. 3A, 5D). The peptide-specific Copiagag antibody shows a similar pattern of enrichment to that of α-CopiaFull at pre- and postsynaptic regions of the NMJ (Fig. 3A). To confirm the specificity of the Copia antibodies, we leveraged the powerful Gal4/UAS system 25, which has been extensively used for precise genetic knockdowns in pre- and/or postsynaptic cells at the NMJ. We designed three UAS-shRNA (aka RNAi) constructs each targeting different regions of copia (Fig. 1B). Using the neuronal driver, C380-Gal4, we observe a substantial reduction of α-Copiagag signal when expressing Copiapol-shRNA and Copia1-shRNA (Fig. 3B, S1C, S1D). As Copia1-shRNA targets the Gag region that Copiagag-siRNA is designed to specifically target we focused on the Copiapol-shRNA strain, to test if targeting different regions of Copia influences Copia expression (Fig. 1B). Not only do we see a decrease of α-Copiagag in presynaptic boutons upon expressing Copiapol-shRNA in neurons, but we also observe a reduction of α-Copiagag in the postsynaptic area abutting synapses (Fig. 3B). See Figure 3K for a definition of the anatomy of a bouton, with pre- and post-synaptic areas highlighted. This strongly suggests the postsynaptic localization of Copiagag is in part due to a Copia protein pool derived from the presynaptic cell (Fig. 3G-H). Consistent with a decrease of Copia protein at the NMJ, using dPCR we tested many of the genotypes used in this work, and found that only flies expressing a Copia-si/shRNA had a significant decrease Copia mRNA expression (Fig. 1E-F).

Due to the enrichment of α-Copiagag, at the larval NMJ, we constructed a single siRNA construct, UAS-Copiagag-siRNA, recognizing the copiagag splice site (Fig. 1B). Copiagag-siRNA is predicted to specifically knock down Copiagag via the mature splice form of Copia. This was consistent with the reduction of α-Copiagag signal at the NMJ when using this Copiagag-siRNA construct as compared to the Copiapol-shRNA constructs when knocked down presynaptically (Fig. 3C). We expressed both Copiagag-siRNA and the Copiapol-shRNA in the post-synaptic muscle, and while we do observe a decrease of α-Copiagag signal in the post-synaptic area, we do not see a decrease of α-Copiagag signal presynaptically (Fig. S4A-E). Suggesting the transfer of Copiagag is pre- to post-synaptic, but Copia mRNA can be affected by post-synaptic RNAi expression.

A reduction of Copia leads to a substantial increase in synaptic bouton number.

The observation that Copiagag is enriched at the NMJ led us to investigate its potential role in synaptic development and plasticity. Synaptic boutons at the NMJ are continuously being formed throughout larval development in correlation to larval muscle growth 26. Thus, a disruption in this NMJ scaling is a measure of developmental synaptic plasticity27. We tested the RNA expression of Copia in larvae of different genotypes and found that only in larvae expressing the RNAi and the driver did we observe an appreciable decrease in levels of Copia (Fig. 1 E and F). Further we observe that upon expressing Copiapol-shRNA with the neuronal C380-Gal4 driver to reduce Copia presynaptically, there is a striking 50% increase in synaptic bouton number compared to the C380-Gal4 driver alone control (Fig. 3D, 3E and 3I). This effect was also seen with the single siRNA construct directed against the *copiagag* mRNA splice site (Fig. 1B, 3D, 3F and 3I). In addition to an enhancement in the number of synaptic boutons, we also observed an increase in “hyperbudding, which we define as the presence of three or more boutons budding off from a central, larger (parent) bouton, upon presynaptic expression of either Copiapol-shRNA or Copiagag-siRNA (Fig. 3E, 3F and 3J).

Copia Mediated Off-target Genic regulation.

It has been proposed that TEs regulate physiological functions through intermediate chimeras, whereby TEs co-splice with nearby genes 28. If a copia co-spliced fragment has a function at the Drosophila NMJ, then repressing Copia may affect NMJ development and/or function through a chimeric intermediate. We have taken several steps to investigate this possibility. As with other RNAi constructs directed against a canonical gene, we created several RNAi constructs that were designed to target Copia specifically. Besides making multiple RNAi constructs targeting different regions of Copia, during design (Fig 1B.), we searched for fragments of copia in the annotated *Drosophila* genome. We found 38 of them being full length, a number consistent with previous analysis 29. We searched entire genome for regions that contain either Copia1-shRNA Copiapol-shRNA and found besides matches to both RNAi constructs in each of the 38 seeds (or 76 matches to Copia seeds in total), another 21 matches to non-seed fragments of Copia scattered throughout the genome. We later determined if the resulting chimeric sequences contained Copiagag-siRNA, as this RNAi construct is too short to effectively search for in the entire genome. No mature mRNA transcript contained any matches to either Copia1-shRNA or Copiapol-shRNA, let alone both.

We next looked for chimeric sequences of Copia that might be present in immature mRNA transcripts. As both Copia1-shRNA Copiapol-shRNA have similar phenotypes in terms of reducing Copiagag expression at the NMJ and similar increases in synaptic boutons and hyberbudding (Fig. S1C-F), when searching for Copia containing chimeras we set the threshold to include at a minimum both those RNAi sequences. Searching the *Drosophila* genome we found 19 intergenic mapping regions that contained at least one of the shRNA sequences. Only one immature transcript on the Y-chromosome, WD40 Y, contained matches to both Copia1-shRNA and Copiapol-shRNA. This transcript contains the splice site that would give rise to Copiagag-siRNA and the α-Copiagag peptide, however, the WD40 Y gene is antisense to this Copia insert in the first intron of WDY (Fig. 1B). In addition, we carried out the phenotypic analysis in female larvae lacking a Y-chromosome, as such it is unlikely that WD40 Y reduction via a Copia-WD40 Y chimera can explain the phenotypes we observe.

To address the presence of unannotated chimeras we used long-RNA sequencing (Nanopore). We found that 60% of all Copia reads mapped to full length seeds (Fig. S5A). However, while we did detect 41 chimeras with significant matches to both Copia and canonical genes, only 2 of these chimeras had a significant match to both Copia1-shRNA and Copiapol-shRNA: Therefore, the vast majority of Copia mapping reads do not map to chimeric transcripts containing fragments of known genes that are capable of being targeted by our RNAi constructs. One of the chimeras is between Copia and the gene CTP, and is unlikely to have an off-target effect. The chimera is antisense to Copia and CTP, contains intronic sequences of CTP, does not encode for a protein and the nearest Copia seed or Copia fragment with both shRNA sequences is >1Mb away. The other chimera is between Copia and the gene Dark, and is also an unlikely candidate as the Copiagag-siRNA sequence is not in the chimera and the nearest Copia seed is >17Mb away. Neither encode for a chimeric protein, and little of CTP or Dark sequences are present in these hybrids. With no predicted chimeric protein being apparent and being these are one-off reads from only one of 3 repeated libraries, we suspect these are artifacts from library production 30.

Further, we investigated the possibility that RNAi of Copia is somehow spreading to suppress expression of neighboring genes by a yet undiscovered mechanism. To determine this, we investigated the expression levels of genes that are in proximity (within 10kb) to our targeted 38 copia seeds, in the CNS of C380-Gal4 driven RNAi of Copiapol-shRNA or Copiagag-siRNA; compared to the CNS of wildtype larvae. In comparison to Copia, we analyzed the change in 1) all genes, 2) genes in the vicinity (+/- 10kb) of 40 tRNAs, 3) the aforementioned TE Gypsy, and 4) in the vicinity of FW TE seeds. We chose Gypsy as it is an active TE in the Drosophila genome and FW as it is a TE in *Drosophila* with similar genomic representation as Copia. In Copiapol-shRNA, genes near Copia seeds were not significantly changed relative to genes near tRNAs, FW elements, and Gypsy elements. Many of these ‘proximal genes’ showed small increases or decrease in gene expression upon the addition of an RNAi construct, (Fig. 2H), suggesting a general trend not specific to regions close to Copia. However, none of these regions (with the exception of all Refseq genes after expression of copiagag RNAi) showed a statistically significant skew toward a general decrease in gene expression after RNAi. In Copiagag-RNAi and Copiapol-shRNA, genes near Copia seeds were not significantly skewed toward a decrease in gene expression relative to any other class of gene (Fig. 2H). We conclude that there is no pervasive bias towards a change of gene expression near Copia seeds upon RNAi expression relative to genes near other genomic features.

Copia is a negative regulator of acute structural synaptic plasticity.

Our discovery that Copia is a negative regulator of developmental plasticity prompted us to test if Copia also affects rapid activity-dependent bouton formation31 . Dissected larval NMJs acutely stimulated with repetitive spaced cycles of nerve stimulation (High K+; 90mM), or light-evoked stimulation of motor neurons expressing photorhodopsin, show acute new synaptic bouton formation31.These nascent boutons, however, do not have the time for proper development of pre- and postsynaptic structures, and are thus referred to as ghost boutons. In previous work, we found dArc1 mutant NMJs were less responsive to spaced K+ stimulation, which resulted in decreased formation of ghost boutons compared to controls. As expected, K+ stimulation of wildtype control larval NMJs leads to a significant increase in ghost boutons (Fig. 4A and 4E). Given Copiapol-shRNA and Copiagag-siRNA larvae have a larger number of synaptic boutons, we thought it possible that a saturating stimulus may mask any effect in the knockdown larvae (see discussion), thus we carried out subthreshold K+ stimulation31.

Normally in wildtype, 5 cycles of spaced stimulation are required for maximal ghost bouton formation, with only 3 cycles being insufficient. Therefore, we used a 3-cycle as the subthreshold K+ stimulation. As expected, subthreshold stimulation did not induce ghost bouton formation in wildtype controls (Fig. 4B and 4E). However, presynaptic driven (using the C380-Gal4 driver) Copiapol-shRNA or Copiagag-siRNA exhibit significant ghost bouton formation upon this level of stimulation (3X) and the levels of ghost bouton formation were similar to the levels found with 5X cycle- stimulation in wildtype (Fig. 4A, 4C, 4D and 4E). This shows presynaptic reduction of Copia flies have increased acute plasticity in addition to developmental plasticity.

Copia and dArc1 have an antagonistic relationship.

That alterations in Copia and dArc1 levels lead to opposite effects at the NMJ, and both proteins appear to signal through the ViSyToR pathway, i.e., can form capsids that binds to their RNA and are transferred across the NMJ through EVs, led us to ask whether the two proteins have antagonistic interactions. This was tested by examining the effects of decreasing Copia levels on dArc1 localization and vice-versa. A presynaptic reduction using Copiapol-shRNA or Copiagag-siRNA leads to a large accumulation of α-dArc1 signal both pre- and postsynaptically (Fig. 5A-C and 5F-G). Additionally, in presynaptic dArc1-RNAi flies, there is an increase of α-Copiagag signal in the pre- and postsynaptic compartments (Fig. 5D-E and 5H-I). Consistent with an increase of dArc1 protein at the NMJ in larvae expressing Copia-RNAi constructs we see an increase of dArc1 mRNA in the pre- and post-synaptic tissue as determined by dPCR (Fig S1A and B).

Copia is a predominant regulator of plasticity.

As dArc1 and Copia have an inverse relationship, in that a reduction of one at the NMJ leads to an increase of the other, we assessed if dArc1 and Copia genetically interacted to determine which is prevalent or upstream of the other. We reduce Copia using Copiapol-shRNA and Copiagag-siRNA lines in a dArc1 null mutant background. We observe a substantial decrease in bouton formation in dArc1 null mutants, consistent with our previous studies (Fig. 6E1 and 6H) (Ashley et al 2018). Reductions of Copia in neurons in a dArc1 null background results in a substantial increase in bouton numbers (Fig. 6D1-G1 and 6H) as well as large increases in hyperbudding (Fig. 6D2-G2 and 6I). This is consistent with Copia having a prevalent role at the NMJ (Fig. 6C).

**Discussion**

In this work we show evidence for a role of the ty1-copia family of transposons, Copia, in the regulation of structural synaptic plasticity at the Drosophila larval NMJ. Copiagag, a spliced form of Copia, is enriched at the NMJ, likely transfers in a pre- to postsynaptic manner in EVs, and when knocked down, leads to abnormal phenotypes at the NMJ. Specifically, a significant increase in the number of boutons, suggesting that Copia is an inhibitor of synaptic formation. Further, in larvae where Copia was downregulated, a subthreshold stimulation of potassium was sufficient to induce a significant change in activity-dependent bouton formation, consistent with a role of Copia in inhibiting activity-dependent plasticity. We also show that Copia interacts genetically in a predominant role with dArc1, a master regulator of plasticity. Taken together this is the first evidence that a TE is a potent regulator of structural synaptic plasticity.

We found there are many similarities between dArc1 and Copia. Both had the ability to spontaneously form capsids when the gag proteins were expressed in bacteria, both were loaded into extracellular vesicles, both proteins had the ability to bind to their own RNA, and both were able to be transferred from neuron to muscle, becoming enriched at the postsynaptic region of the NMJ. However, there were also many notable differences. The synthetic Copia capsids we observed in this study were larger than synthetic dArc1 capsids. This is likely due to the larger size of the Copia transcript as it has been demonstrated viral transcript size can drive capsid size 25,32,33. Another difference was the phenotype observed at the NMJ when Copia or dArc1 was downregulated in neurons. A reduction of CopiaFull and Copiagag in neurons resulted in a striking increase in both synaptic bouton number during development and rapid activity dependent synaptic bouton formation.Interestingly, Copia and dArc1 had an antagonistic relationship, with Copia being genetically dominant, providing evidence the two capsid-encoding genes interact genetically to mediate plasticity at the fly NMJ. This effect on synaptic formation is one of the first, if not the first, description of a physiological role of a TE in neurons. Further, it reveals the finding that a TE interacts with a canonical gene.

Several questions remain. Are other capsid-encoding proteins transferred across cells in the nervous system or other tissues? Recently, the engineering and use of the mammalian capsid-like protein, peg10, to take advantage of the ViSyToR pathway to transfer RNA cargo, provides growing evidence that this pathway is relevant beyond the Drosophila NMJ 34. While not proof that peg10 is needed for moving RNA from one cell to another, it is strong evidence other capsid-encoded genes act in a viral-like manner. For instance, the retrotransposon Cer1 in *C. elegans* has been shown to form capsids and may have a role in pathogen avoidance35. These studies along with our discovery that a TE, Copia, regulates synaptic plasticity, raises the highly likely possibility that dArc1 was an initial member of a family of capsid-encoding genes that regulate cellular functions at neurons and beyond.

While the interaction between Copia and dArc1 does give a glimpse into the genetic scaffold that underlies plasticity, it is still generally not known how these molecules function at a molecular level. However, the Copia mutant phenotype may give hints. The most consistent phenotype in Copia mutants is hyperbudding, which is often characterized as an overgrowth of synapses. There are several mutations and pathways associated with hyperbudding, including retrograde TGF-β/BMP signaling. Of note is the TGF-β/BMP signal depends on presynaptic endocytosis 36. Endocytosis is the first step in endosome formation. The resulting internalized endosomes can be recycled via a multivesicular body back to cell membrane, resulting in the release of extracellular vesicles. As we discovered both dArc1 and Copia are present in extracellular vesicles, it would be interesting to test how endocytosis affects Copia transport across the synapse, and if in this process, one is regulating the other.

What other ways could a TE, such as Copia regulate synaptic plasticity? One suggested mechanism is through an ‘off-target’ or ‘chimeric’ model, something we attempted to address in this work. We looked for chimeric sequences in the genome that contained Copia and canonical genes, none could be targeted by all the RNAi constructs we used. We further did long NGS and found while chimeras do exist, consistent with other publications, but again the chimeras we find are not targeted by the RNAi constructs we employ, the ones that are, are not very common, and not near known seeds of Copia, which suggests that these chimeras are a product of deep sequencing noise. Alternatively, another model is via ‘curing an infection’, such that a knock-down of Copia leads to healthier cells as these cells are not burdened with a viral-like infection. No data in this work conflicts with such a model, however, as TEs are passed through the germline, it is unclear what evolutionary advantage a TE would have to be somatically infectious and repress plasticity. This might be testable, if increased ‘infections’ of neurons by a TE led to a reduction of plasticity, then the prediction would be that a reduction of Copia would cause a decrease in lifespan and/or egg laying, or some other measure of fecundity.

There are many examples of TE domestication, including dArc1, whereby a TE fragment is selected through evolution for having a beneficial role for the host. There are few examples, however, where an entire transposon is domesticated, not a fragment. With the exceptions of the aforementioned Cer1 *of C. elegans* and Line-1 elements which have a role in early mouse embryonic development35,37. While it has been long speculated that TEs do have a role in neuronal development, and there is data that expression of Line-1 in mammalian brains is developmentally regulated, a specific role for Line-1 or any TE in neurons has not been identified38-40. One underlying premise of the above studies is that the insertion of a TE into the genome has an indirect effect that influences gene expression/function. This would be consistent with the somatic mosaicism described by Barbara McClintock15. However, the studies presented here do not address a potential regulation of synaptic plasticity through the insertion of Copia into the genome. It is possible that upon crossing a synaptic bouton, Copia could insert into the muscle genome, which in turn regulates synaptic development and plasticity. Regardless, the role of a TE in synaptic development needs to be further explored, not only to understand synaptic plasticity, but to understand the potential functions of a large part of eukaryotic genomes arguably mislabeled as “junk”.

**METHODS**

Experimental Model and Subject Details

The following fly lines were used in generating the presented data; UAS-Copiapol-shRNA (see below), UAS-CopiaGag-siRNA (see below), UAS-dArc1-RNAi2 37, w; dArc1esm18 (RRID:BDSC\_37530, Bloomington *Drosophila* stock center, BDSC), y[1] w[67c23]; P{y[+t7.7]=CaryP}attP2 (RRID: RRID:BDSC\_8622, Bloomington *Drosophila* stock center, BDSC), Canton-S (1, BDSC), C380-Gal4 41 and C57-Gal441 . Female third-instar larvae were used for all NMJ dissections.

Fly Husbandry  
All flies were raised on low yeast molasses formulation *Drosophila* food at either 25°C or 29°C (Gal4/RNAi crosses).

Constructs

For copiapol-shRNA and other dsRNA constructs, the insert was synthesized (see attached construct sequence file), then cloned into pwallium10-roe as described in Ni et al 201142. For the copiagag siRNA, a forward and reverse primer were synthesized (see attached construct sequence file), annealed and cloned int pwallium20 as described in Ni et al 201142.

Immunocytochemistry and Antibodies

*Drosophila melanogaster* third instar larva body wall muscles were dissected in calcium-free saline and fixed in either Bouin’s fixative (0.9% (v/v) picric acid, 5% (v/v) glacial acetic acid, 9% (w/v) formaldehyde) or 4% (w/v) paraformaldehyde in 0.1M phosphate buffer, pH 7.2. Fixed samples were washed and permeabilized in PBT (0.1M phosphate buffer; 0.2% (v/v) Triton X-100) and incubated in a primary antibody overnight at 4°C. The samples were then washed three times with PBT, incubated with secondary antibodies for two hours at room temperature, washed three times, and then mounted in Vectashield Hardset Mounting Media (Vector Laboratories Inc.). The following antibodies were used: rabbit anti-CopiaFull, 1:1000 (see below), rabbit anti-CopiaGag, 1:5000 (see below), rabbit anti-dArc1, 1:50043, rabbit anti-DLG, 1:40,000 44and mouse anti-DLG, 1:200 (Developmental Studies Hybridoma Bank (DSHB), 4F3). DyLight-conjugated and Alexa Fluor-conjugated secondary antibodies were obtained from Jackson ImmunoResearch (DyLight-405-conjugated goat anti-HRP, Alexa Fluor-594-congugated goat anti-HRP, Alexa Fluor-488-congugated donkey anti-Rabbit, Alexa Fluor-594-congugated goat anti-rabbit, Alexa Fluor-647-congugated goat anti-Mouse) and were used at 1:200, as described above.

CopiaFull antibodies were generated against a Copia antigen (***see figure 1***) by immunizing rabbits with then entire Copiagag protein (Pocono Rabbit Farm and Laboratory), while the Copiagag antibodies were generated against a Copia peptide antigen (***see figure 1***) by immunizing rabbits with the peptide LMVVKNSENQLADIC (GenScript).

Activity Paradigm

Potassium stimulations were carried out as described in31. Larva were dissected in low-calcium (0.1mM) HL3 saline45, then pulsed with a series of high potassium (90mM) saline; each pulse was spaced out by a 15-minute rest period in low-calcium HL3 saline. The 5-cycle potassium stimulation consisted of three 2-minute pulses, one 4-minute pulse and one 6-minute pulse; followed by a final 15-minute rest. The 3-cycle, subthreshold, potassium stimulation consisted of three 2-minute pulses followed by a 15-minute rest period. Following the 90-minute pulse-rest cycle, samples were fixed with 4% paraformaldehyde and processed for immunocytochemistry as described above.

Confocal Microscopy and Signal Intensity Measurements

Z-stacked images were acquired using a Zeiss LSM 800 confocal microscope equipped with a Zeiss 63X Plan-Apochromat 1.40 NA DIC M27 oil immersion objective and a Zeiss 40X Plan-Apochromat 1.30 NA DIC (UV) VIS-IR M27 oil immersion objective. After image acquisition with identical settings, the images were quantified as previously described46. In brief, volumetric measurements of the boutons of interest bound by HRP staining was selected and fluorescence intensity inside was measured using Volocity software (Quorum Technologies Inc). Postsynaptic area was calculated by dilating the presynaptic area by 8 iterations and comparing to DLG staining and the HRP containing volume was subtracted and the intensity within the remaining volume was measured. Intensity was normalized to HRP bouton volume and data normalized to wild type values.

We found that the knockdown of dArc1 α-Copiagag is saturated, to collect data at a linear intensity, we normalized in this case the saturation points in dArc1-siRNA, not wildtype, thus a lighter intensity in controls for this experiment (Fig. S3 C and D). Fluorescent intensity was measured in the subthreshold larvae (Fig. 5 H and I).

RNA Immunoprecipitation

Wild type Drosophila third-instar larvae were dissected, and the CNS and body wall muscles were collected in separate tubes containing RIPA buffer (Abcam) supplemented with protease inhibitors (Roche) and RNase inhibitor (Invitrogen) in a manner previously described43. Similarly, S2 cells were grown to confluency, washed with ice-cold DPBS (Sigma) and resuspended in RIPA buffer. Tissue and cell lysates were homogenized using 0.5mm glass beads at 4°C using a Bullet Blender 24 Gold homogenizer (Next Advance Inc.). Lysates were then centrifuged at 4°C to remove cell debris. Supernatants were precleared against Protein A/G magnetic beads (Pierce), and then incubated overnight at 4°C with either anti-copiafull, anti-copiagag, anti-dArc1 antibodies, or equal amounts of pre-immune serum. Samples were then incubated for 2 hours at 4°C with protein A/G magnetic beads and washed several times with RIPA buffer. For immunoblotting, beads were incubated directly with 4X protein loading buffer (Li-Cor) with 2-Mercaptoethanol (Sigma). For digital PCR, RNA was eluted from the beads with RLT buffer (QIAGEN) supplemented with 2-mercaptoethanol and then purified using the RNeasy mini kit (QIAGEN) for the QIAcube connect (QIAGEN) with DNase digest using RNase-free DNase set (QIAGEN).

Digital PCR (dPCR)

RNA samples were reverse transcribed into cDNA using the Superscript IV first-strand synthesis reaction (Invitrogen) following manufacturer protocol with RNase H digest. The dPCRs were multiplexed in 26K 24-well or 8.5K 96-well QIAcuity nanoplates (QIAGEN) using a QIAcuity system (QIAGEN). For the reactions, either QIAcuity evagreen master mix or probe master mix (QIAGEN) were used with the gene specific primer sets for dArc1, Copiafull, Copiagag, Rpl32 and/or 18S rRNA or their probes (ThermoFisher or IDT). Data was processed in the QIAcuity Software Suite (QIAGEN) where absolute values (copies/µL) were obtained, and normalized expression derived.

Immunoprecipitation

Third-instar wild type Drosophila larvae were dissected in ice-cold Ca2+-free saline, and CNS and body wall muscles were homogenized as above. For S2 cells, they were grown to confluency, washed in ice-cold DPBS (Sigma), resuspended in RIPA buffer (Abcam) supplemented in protease inhibitor cocktails (Roche), and homogenized as above. Lysates were centrifuged at maximum speed at 4°C for 10 minutes. Protein concentration was determined by Qubit protein assay (Invitrogen) in a Qubit 4 fluorometer (Invitrogen). Supernatants were incubated with the respective antibody overnight at 4°C with gentle rotation, the protein-antibody complexes were incubated with protein A/G magnetic beads (Pierce) for an hour at room temperature. They were washed with buffer several times with final wash being in pure water. The magnetic beads were eluted with protein sample buffer at room temperature for 10 minutes with gentle rotation or boiled at 95°C for 10 minutes.

Western Blotting

Immune complexes from RIP and IP experiments were incubated at room temperature or 95°C for 10 min. proteins were separated in Mini-Protean TGX stain-free 4%–20% precast gels (Bio-Rad) under reducing and denaturing conditions. Proteins were transferred to an Immun-Blot LF PVDF membrane (Bio-Rad) on a semi-dry Trans-Blot Turbo transfer system (Bio-Rad), blocked in Intercept blocking buffer (Li-Cor) and incubated with primary antibodies diluted in Intercept antibody diluent (Li-Cor) overnight at 4°C. Blots were washed, incubated with IRDye secondary antibodies (Li-Cor), washed again and finally imaged on a Li-Cor odyssey CLx imaging system.

Copia Capsid Preparation

The Copiagag ORF was cloned into pENTR (Thermo Fisher) and after sequence confirmation was subsequently recombined using LR Clonase (Thermo Fisher) with the pDEST-HisMbp Destination vector 47 to generate His-MBP-Copiagag. Expression and induction were achieved in transformed BL21(DE3) *E. coli* (ThermoFisher)cells grown in LB media. Protein purification under native conditions was performed in Ni-NTA fast start kit (QIAGEN) following manufacturer conditions and dialysed against PBS in a Slide-A-Lyzer dialysis cassette (Thermo Fisher). Soluble protein was diluted to ∼1 mg/ml, cleaved with TEV protease (GenScript) at 30°C, the His-MBP tag and the His tagged TEV were removed by binding to a Ni-NTA column (QIAGEN) or by using HisPur Ni-NTA magnetic beads (Thermo Scientific)..

Transmission Electron Microscopy of Copia Capsid

Copia capsids were allowed to form in PBS and then examined using negative staining. Capsids were fixed in EM grade 2% paraformaldehyde (EMS) overnight at 4°C. 5μL of fixed capsids were spotted onto formvar coated copper grids (EMS). After 5 min absorption, grids were wicked off in Whatman filter paper (GE Healthcare), rinsed with PBS and post-fixed with 1% glutaraldehyde for 5 min. Samples were washed with water and then counter-stained with 2% w/v uranyl acetate (EMS) and imaged on an FEI Tecnai 12 Spirit electron microscope (FEI Company) equipped with a Gatan 4K camera at 120kv.

Quantification of Copia Capsids

Capsids were quantified using Tunable resistive pulse sensing (TRPS) for size and concentration using the Exoid system (Izon Sciences) following manufacturer recommendations. Briefly, the nanopore was set up, characterized, and wetted. Nanopore coating for biological samples was done with the Izon coating solution (Izon Sciences). Measurement of the samples was taken alongside calibration readings and the data analyzed for multi-pressure analysis using the Izon data suite (Izon Sciences).

Isolation and Quantification of EVs from S2 Cells

Extracellular vesicles (EVs) were isolated from S2 cells cultured in serum-free medium at 22°C. EVs-containing media was first centrifuged at 500 x g for 5 min to pellet the cells, the supernatant was then spun at 2000 × g for 10 min at 4°C to eliminate cell debris, and to retain small EVs the samples were further centrifuged at 10,000 × g for 30 min at 4°C. The supernatants were filtered with a 0.22 µm PES filtration unit (EMD Millipore), the samples were concentrated with a Centricon centrifugal filter (EMD Millipore) and the EV-rich filtride recovered. The samples were then purified by size exclusion chromatography using qEV isolation columns (Izon Sciences) on an automatic fraction collector (Izon Science), the fractions were then pooled based on protein concentration and using Amicon centrifugal filters (EMD Millipore) concentrated the samples. Samples for quantification were diluted in PBS and as previously described analyzed for size and concentration using the Exoid TRPS system (Izon Science). Unused samples were stored at -80°C.

Transmission Electron Microscopy of S2 Cells EVs

EVs were fixed in 2% paraformaldehyde overnight at 4°C and 5μL was spotted onto formvar coated grids for 5 min. The grids were wicked using Whatman filter paper, rinsed with PBS and post-fixed with 1% glutaraldehyde for 5 min. The protocol was completed as described above for capsids.

S2 Cells Derived EVs Immuno-Electron Microscopy

Samples were prepared as previously described43. In brief, the EV preparations were fixed overnight at 4°C in 2% PFA (EMS) and 5μL of sample applied to formvar coated gold grids (EMS) and incubated for 5 min. Grids were wicked on Whatman #50 filter paper (GE Healthcare) after which they were washed in 100mM Tris followed by additional washes in 100mM Tris + 50mM Glycine. Grids were blocked for 10 min in blocking buffer (EMS) and either incubated in Tris (control) or lysed with 0.05% saponin in Tris. After washes in Tris, they were incubated in primary antibody for 1hr, washed in Tris, and then incubated with anti-rabbit and/or anti-mouse conjugated to 10nm, 15nm or 18nm gold secondary antibodies (EMS). Grids were washed, post fixed with 1% glutaraldehyde, washed in water, and finally negative stained with 1% uranyl acetate for 30 s. Grids were imaged on an FEI Tecnai 12 Spirit equipped with a Gatan 4K camera.

Quantification and Statistical Analysis

Statistical analysis for single comparison’s was performed using a Student’s t test while multiple comparison’s with experimental groups utilized a one-way analysis of variance (ANOVA) with the appropriate post hoc test. ∗, p < 0.05; ∗∗, p < 0.001; ∗∗∗, p < 0.0001. Raw data files were processed with Excel (Microsoft) and data analysis for statistical significance utilized GraphPad Prism version 9.5.0 (GraphPad Software).

**Author contributions**

PM, AS, VB, and TT designed experiments. AL, PM, AS, and TT performed the experiments. PM, VB, AS, and TT interpreted the results. VB and TT contributed to writing the manuscript. VB and TT directed the project.

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**BIBLIOGRAPHY AND REFERENCES CITED**

1 Budnik, V. & Thomson, T. Structure of an Arc-ane virus-like capsid. *Nat Neurosci* **23**, 153-154 (2020). <https://doi.org:10.1038/s41593-019-0580-3>

2 Ashley, J. *et al.* Retrovirus-like Gag Protein Arc1 Binds RNA and Traffics across Synaptic Boutons. *Cell* **172**, 262-274 e211 (2018). <https://doi.org:10.1016/j.cell.2017.12.022>

3 Pastuzyn, E. D. *et al.* The Neuronal Gene Arc Encodes a Repurposed Retrotransposon Gag Protein that Mediates Intercellular RNA Transfer. *Cell* **172**, 275-288 e218 (2018). <https://doi.org:10.1016/j.cell.2017.12.024>

4 de Koning, A. P., Gu, W., Castoe, T. A., Batzer, M. A. & Pollock, D. D. Repetitive elements may comprise over two-thirds of the human genome. *PLoS Genet* **7**, e1002384 (2011). <https://doi.org:10.1371/journal.pgen.1002384>

5 Rodic, N. & Burns, K. H. Long interspersed element-1 (LINE-1): passenger or driver in human neoplasms? *PLoS Genet* **9**, e1003402 (2013). <https://doi.org:10.1371/journal.pgen.1003402>

6 Routh, A., Domitrovic, T. & Johnson, J. E. Host RNAs, including transposons, are encapsidated by a eukaryotic single-stranded RNA virus. *Proc Natl Acad Sci U S A* **109**, 1907-1912 (2012). <https://doi.org:10.1073/pnas.1116168109>

7 Payer, L. M. & Burns, K. H. Transposable elements in human genetic disease. *Nat Rev Genet* **20**, 760-772 (2019). <https://doi.org:10.1038/s41576-019-0165-8>

8 Belyayev, A. Bursts of transposable elements as an evolutionary driving force. *J Evol Biol* **27**, 2573-2584 (2014). <https://doi.org:10.1111/jeb.12513>

9 Bourque, G. *et al.* Ten things you should know about transposable elements. *Genome Biol* **19**, 199 (2018). <https://doi.org:10.1186/s13059-018-1577-z>

10 Jones, J. M. & Gellert, M. The taming of a transposon: V(D)J recombination and the immune system. *Immunol Rev* **200**, 233-248 (2004). <https://doi.org:10.1111/j.0105-2896.2004.00168.x>

11 Chen, H. *et al.* The Exonization and Functionalization of an Alu-J Element in the Protein Coding Region of Glycoprotein Hormone Alpha Gene Represent a Novel Mechanism to the Evolution of Hemochorial Placentation in Primates. *Mol Biol Evol* **34**, 3216-3231 (2017). <https://doi.org:10.1093/molbev/msx252>

12 Kordyukova, M., Olovnikov, I. & Kalmykova, A. Transposon control mechanisms in telomere biology. *Curr Opin Genet Dev* **49**, 56-62 (2018). <https://doi.org:10.1016/j.gde.2018.03.002>

13 Zhang, H. & Bramham, C. R. Arc/Arg3.1 function in long-term synaptic plasticity: Emerging mechanisms and unresolved issues. *Eur J Neurosci* (2020). <https://doi.org:10.1111/ejn.14958>

14 Finnegan, D. J., Rubin, G. M., Young, M. W. & Hogness, D. S. Repeated gene families in Drosophila melanogaster. *Cold Spring Harb Symp Quant Biol* **42 Pt 2**, 1053-1063 (1978). <https://doi.org:10.1101/sqb.1978.042.01.106>

15 McClintock, B. The significance of responses of the genome to challenge. *Science* **226**, 792-801 (1984).

16 Flavell, A. J., Ruby, S. W., Toole, J. J., Roberts, B. E. & Rubin, G. M. Translation and developmental regulation of RNA encoded by the eukaryotic transposable element copia. *Proc Natl Acad Sci U S A* **77**, 7107-7111 (1980). <https://doi.org:10.1073/pnas.77.12.7107>

17 Flavell, A. J. & Ish-Horowicz, D. The origin of extrachromosomal circular copia elements. *Cell* **34**, 415-419 (1983). <https://doi.org:10.1016/0092-8674(83)90375-6>

18 Flavell, A. J. Role of reverse transcription in the generation of extrachromosomal copia mobile genetic elements. *Nature* **310**, 514-516 (1984). <https://doi.org:10.1038/310514a0>

19 Lefebvre, F. A. *et al.* Comparative transcriptomic analysis of human and Drosophila extracellular vesicles. *Sci Rep* **6**, 27680 (2016). <https://doi.org:10.1038/srep27680>

20 Yoshioka, K. *et al.* Virus-like particle formation of Drosophila copia through autocatalytic processing. *EMBO J* **9**, 535-541 (1990).

21 Konvalinka, J., Krausslich, H. G. & Muller, B. Retroviral proteases and their roles in virion maturation. *Virology* **479-480**, 403-417 (2015). <https://doi.org:10.1016/j.virol.2015.03.021>

22 Gazda, L. D., Joone Matuz, K., Nagy, T., Motyan, J. A. & Tozser, J. Biochemical characterization of Ty1 retrotransposon protease. *PLoS One* **15**, e0227062 (2020). <https://doi.org:10.1371/journal.pone.0227062>

23 Perilla, J. R. & Gronenborn, A. M. Molecular Architecture of the Retroviral Capsid. *Trends Biochem Sci* **41**, 410-420 (2016). <https://doi.org:10.1016/j.tibs.2016.02.009>

24 Zhao, G. *et al.* Mature HIV-1 capsid structure by cryo-electron microscopy and all-atom molecular dynamics. *Nature* **497**, 643-646 (2013). <https://doi.org:10.1038/nature12162>

25 Brand, A. H. & Perrimon, N. Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* **118**, 401-415 (1993).

26 Budnik, V., Zhong, Y. & Wu, C. F. Morphological plasticity of motor axons in Drosophila mutants with altered excitability. *J Neurosci* **10**, 3754-3768 (1990).

27 Menon, K. P., Carrillo, R. A. & Zinn, K. Development and plasticity of the Drosophila larval neuromuscular junction. *Wiley Interdiscip Rev Dev Biol* **2**, 647-670 (2013). <https://doi.org:10.1002/wdev.108>

28 Treiber, C. D. & Waddell, S. Transposon expression in the Drosophila brain is driven by neighboring genes and diversifies the neural transcriptome. *Genome Res* **30**, 1559-1569 (2020). <https://doi.org:10.1101/gr.259200.119>

29 Kaminker, J. S. *et al.* The transposable elements of the Drosophila melanogaster euchromatin: a genomics perspective. *Genome Biol* **3**, RESEARCH0084 (2002). <https://doi.org:10.1186/gb-2002-3-12-research0084>

30 Boers, S. A., Jansen, R. & Hays, J. P. Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory. *Eur J Clin Microbiol Infect Dis* **38**, 1059-1070 (2019). <https://doi.org:10.1007/s10096-019-03520-3>

31 Ataman, B. *et al.* Rapid activity-dependent modifications in synaptic structure and function require bidirectional Wnt signaling. *Neuron* **57**, 705-718 (2008). <https://doi.org:10.1016/j.neuron.2008.01.026>

32 Hagan, M. F. Modeling Viral Capsid Assembly. *Adv Chem Phys* **155**, 1-68 (2014). <https://doi.org:10.1002/9781118755815.ch01>

33 Perlmutter, J. D., Qiao, C. & Hagan, M. F. Viral genome structures are optimal for capsid assembly. *Elife* **2**, e00632 (2013). <https://doi.org:10.7554/eLife.00632>

34 Segel, M. *et al.* Mammalian retrovirus-like protein PEG10 packages its own mRNA and can be pseudotyped for mRNA delivery. *Science* **373**, 882-889 (2021). <https://doi.org:10.1126/science.abg6155>

35 Moore, R. S. *et al.* The role of the Cer1 transposon in horizontal transfer of transgenerational memory. *Cell* **184**, 4697-4712 e4618 (2021). <https://doi.org:10.1016/j.cell.2021.07.022>

36 O'Connor-Giles, K. M., Ho, L. L. & Ganetzky, B. Nervous wreck interacts with thickveins and the endocytic machinery to attenuate retrograde BMP signaling during synaptic growth. *Neuron* **58**, 507-518 (2008). <https://doi.org:10.1016/j.neuron.2008.03.007>

37 Percharde, M. *et al.* A LINE1-Nucleolin Partnership Regulates Early Development and ESC Identity. *Cell* **174**, 391-405 e319 (2018). <https://doi.org:10.1016/j.cell.2018.05.043>

38 Muotri, A. R. L1 Retrotransposition in Neural Progenitor Cells. *Methods Mol Biol* **1400**, 157-163 (2016). <https://doi.org:10.1007/978-1-4939-3372-3_11>

39 Muotri, A. R. *et al.* L1 retrotransposition in neurons is modulated by MeCP2. *Nature* **468**, 443-446 (2010). <https://doi.org:10.1038/nature09544>

40 Muotri, A. R., Zhao, C., Marchetto, M. C. & Gage, F. H. Environmental influence on L1 retrotransposons in the adult hippocampus. *Hippocampus* **19**, 1002-1007 (2009). <https://doi.org:10.1002/hipo.20564>

41 Budnik, V. Synapse maturation and structural plasticity at Drosophila neuromuscular junctions. *Curr Opin Neurobiol* **6**, 858-867 (1996).

42 Ni, J. Q. *et al.* A genome-scale shRNA resource for transgenic RNAi in Drosophila. *Nat Methods* **8**, 405-407 (2011). <https://doi.org:10.1038/nmeth.1592>

43 Ashley, J. *et al.* Retrovirus-like Gag Protein Arc1 Binds RNA and Traffics across Synaptic Boutons. *Cell* **172**, 262-274.e211 (2018). <https://doi.org:10.1016/j.cell.2017.12.022>

44 Koh, Y. H., Popova, E., Thomas, U., Griffith, L. C. & Budnik, V. Regulation of DLG localization at synapses by CaMKII-dependent phosphorylation. *Cell* **98**, 353-363 (1999).

45 Stewart, B. A., Atwood, H. L., Renger, J. J., Wang, J. & Wu, C. F. Improved stability of Drosophila larval neuromuscular preparations in haemolymph-like physiological solutions. *J Comp Physiol A* **175**, 179-191 (1994).

46 Ramachandran, P., Barria, R., Ashley, J. & Budnik, V. A critical step for postsynaptic F-actin organization: regulation of Baz/Par-3 localization by aPKC and PTEN. *Dev Neurobiol* **69**, 583-602 (2009). <https://doi.org:10.1002/dneu.20728>

47 Nallamsetty, S., Austin, B. P., Penrose, K. J. & Waugh, D. S. Gateway vectors for the production of combinatorially-tagged His6-MBP fusion proteins in the cytoplasm and periplasm of Escherichia coli. *Protein Sci* **14**, 2964-2971 (2005). <https://doi.org:10.1110/ps.051718605>