Living Synaptic Vesicle Marker: Synaptotagmin-GFP

Yong Q. Zhang, Christopher K. Rodesch, and Kendal Broadie*

Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235-1634

Received 4 June 2002; Accepted 17 July 2002

Synapses are the site of chemical communication between neurons and between neurons and muscles. The synaptic vesicle (SV) is a prominent presynaptic organelle which contains chemical neurotransmitters and fuses with the plasma membrane to mediate neurotransmission. There are about 50 or so synaptic proteins which are either integral vesicle membrane proteins (e.g., synaptotagmin, syt; and synaptobrevin, syb) or vesicle-associated proteins (e.g., cysteine string protein, CSP; Fernandez-Chacon and Sudhof, 1999). We have transformed an enhanced GFP (enhanced GFP) fusion protein, the fluorescence pattern of which colocalizes with native SV proteins at synapses, suggesting that the syt-eGFP fusion protein is correctly localized as an integral SV protein and therefore a good SV marker in living synapses. We demonstrate that the syt-eGFP line can be used to study SV dynamics in vivo by fluorescence recovery after photobleach (FRAP).

The syt-eGFP fusion was constructed as shown in Figure 1. The eGFP carries double substitution of Phe 64 to Leu and Ser 65 to Thr and fluoresces 35-fold more intensely than wild-type GFP when excited at 488 nm, based on spectral analysis of equal amounts of soluble protein (Cormack et al., 1996). Four syt-eGFP transgenic lines were generated; one with insertion on the X chromosome, two on the second chromosome, and one on the third chromosome. All of these lines produced clear fluorescence when crossed to a pan-neuronal GAL4 driver (elav-GAL4, see Fig. 2) or a subset neuronal GAL4 driver 4G-GAL4 (data not shown). 4G-GAL4 is identified from an enhancer trap screen for neuronal specific genes; it starts expression at late embryogenesis pan-neuronally, but in a subset of motor neurons in the third instar larvae, and enriched in mushroom body in adult brain. The eGFP-positive animals, from embryos to adults, can be readily recognizable under a fluorescence dissecting scope. Stocks with expression of syt-eGFP in all neurons (recombinant chromosome carrying both elav-GAL4 and syt-eGFP on the X chromosome) or subset of neurons (recombinant chromosome carrying both 4G-GAL4 and syt-eGFP on the second chromosome) were established.

Multiple lines of evidence indicate that syt-eGFP is present in SVs, with expression similar to the native syt (Fig. 2). First, syt-eGFP is highly enriched in the neuropil region of the ventral nerve cord (VNC) of the embryo (data not shown) and larva (Fig. 2A, left), as well as in the axonal lobes of the larval mushroom body (Fig. 2A, right). These neuropil regions are densely packed with neuronal synapses. Second, at neuromuscular junction (NMJ) synapses, where we have higher resolution of single synaptic boutons, the syt-eGFP pattern perfectly matches the staining pattern seen with antibodies against SV-associated proteins (Fig. 2B). This suggests that syt-eGFP is tightly linked to SVs. Third, to further

* Correspondence to: Kendal Broadie, Department of Biological Sciences, Vanderbilt University, Nashville, TN 37255-1634.
E-mail: brodie@biology.utah.edu

Present address for Christopher K. Rodesch: University of Utah, Cell Imaging Facility, Bldg. 585, 40 N. 2030 E., Salt Lake City, UT 84112.

Contract grant sponsor: National Institutes of Health; Contract grant number: HD40654 (to KB).

DOI: 10.1002/gene.10144
FIG. 2

A

VNC

Brain

cell bodies
dendrites
axon lobes
peduncles

B

Syt-GFP

α-CSP

Merged

C

α-Syt

α-CSP

Merged

FIG. 2
demonstrate that syt-eGFP is specifically present in SVs, we took advantage of a dynamin mutant shibh to drive fusion in the absence of vesicle recycling (Estes et al., 1996). shibh mutants block SV endocytosis at restrictive temperature (35°C), causing SV proteins to trap in the plasma membrane. We made a stock that carries shibh, elav-GAL4, and syt-eGFP. As shown in Figure 2C, syt-eGFP is redistributed to the periphery of synaptic boutons at the restrictive temperature, consistent with it being restricted to SVs. Based on the enrichment of syt-eGFP in synaptic regions of CNS, identical localization with endogenous SV proteins at NMJ synapses, and the peripheral redistribution of syt-eGFP at shibh NMJ synapses at restrictive temperature, we conclude that syt-eGFP is restricted to SVs, similar to the native syt.

While synaptic activity can be directly measured using electrophysiology and synaptic vesicle recycling can be studied using uptake of fluorescent marker dyes (Ryan et al., 1996), both of these techniques require that vesicles take part in a full recycle of exocytosis and endocytosis. Therefore, mutations that severely alter the processing of SVs or their mobilization will contain SV subpopulations that are inaccessible to these conventional techniques. Genetically engineered syt-eGFP-labeled SV markers, however, can be used to visualize SVs in mutants that have severe effects on SV release, clustering, transport, or recycling. Therefore, these markers complement activity-dependent dye loading and electrophysiology, techniques that are both better suited for study of mutations which have relatively mild effects on synaptic activity. Moreover, FRAP analyses of syt-eGFP-labeled SVs is best used to measure the dynamic intermixing of fluorescently labeled SVs with photobleached compartments within synaptic boutons (Fig. 3), which cannot be achieved by classical electrophysiological assays or dye loading.

In addition to the syt-eGFP lines, we have also made syb-eGFP transgenic lines (see Fig. 1). In total, eight independent transgenic lines with insertions on all major chromosomes (the X, the second, and the third chromosomes) were obtained. All eight lines, when crossed to either elav-GAL4 or 4G-GAL4, produce eGFP-positive animals. While syb-GFP(S65T) transgenic flies have become available recently (Estes et al., 2000), we expect that the syb-eGFP line described here will be better suited for some experiments, e.g., biogenesis of SVs, as eGFP matures faster to the fluorescent form and folds more efficiently than GFP(S65T) (Cormack et al., 1996). In parallel experiments, we observed that the fluorescence of syt-eGFP is consistently brighter than that of syb-eGFP. One plausible explanation is that the fluorescence of eGFP is dimmer at lower pH (Tsien, 1998), the lumen of vesicles is acidic (pH 5.6; Miesenbock et al., 1998), and therefore it quenches the fluorescence of eGFP, as the eGFP end of syb-GFP is inside the lumen (see Fig. 1 Legend). The eGFP end of syt-eGFP, however, is located at the cytoplasmic side of the synaptic membrane (see Fig. 1 Legend). Therefore, the fluorescence of syt-eGFP will not be affected by the reduced pH in the vesicle lumen.

We observed that animals with expression of syt-eGFP driven by 4G-GAL4 are fully viable, whereas animals carrying 4G-GAL4, syb-eGFP are lethal at the pupal stages. The reason for the syb-eGFP lethality is currently unknown. One possibility is that syb is a component of the core complex of membrane fusion (Fernandez-Chacon and Sudhof, 1999) and the overexpression of syb in the form of syb-eGFP in neurons might have a dominant-negative effect on the core complex. We expect that the novel SV marker of syt-eGFP with increased intensity and less deleterious effects will prove to be an important tool for addressing a number of essential neurobiological questions: biogenesis of SVs, axonal transport and dynamics of SVs, synaptogenesis, and synaptic development and function in flies. The syt-eGFP line is especially suited for imaging of live samples, as illustrated in Figure 3.
3. This reagent will be applicable to mammals with minor modification.

ACKNOWLEDGMENTS

We thank Sean Speese for the pUAST-eGFP construct on which the syt- and syb-eGFP fusions were built, Michael Bastiani for assistance with confocal imaging, and Bob Renden for comments on the manuscript. Y.Z. was supported by a postdoctoral fellowship from the FRAXA Research Foundation. K.B. is supported by an EJLB Scholarship.

LITERATURE CITED


