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GRASP the concept

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Researchers map synaptic partners by reconstituting complementary fragments of fluorescent proteins tethered to pre- and postsynaptic neurons.

If it took nearly 20 years to map neural circuits in *C. elegans*, how long will it take to map the human 'connectome'? In complex nervous systems, synapses can be so densely packed that it is difficult to distinguish synaptic partners even with the help of synaptic markers. Now Feinberg *et al.* report a technique to map synapses using complementary fragments of green fluorescent protein (GFP) in a recent article in *Neuron*.

In most central nervous system synapses, less than 100 nm separates pre- and postsynaptic membranes. This extracellular space can be bridged by pairs of transmembrane proteins on pre- and postsynaptic cells. The authors designed a transynaptic expression system in which only apposed neurons reconstitute a divided fluorescent label called GFP reconstitution across synaptic partners (GRASP).

GFP forms a -barrel structure, which is a -sheet that folds into a closed structure with the first and last residues bonded together. The GFP -barrel has 11 strands. The first 214 amino acids of GFP include the first 10 strands (GFP1-10), and the next 16 residues form strand 11 (GFP11). Without the complementary fragment, both GFP1-10 and GFP11 are stable, soluble and nonfluorescent. *C. elegans* lacks ligands for CD4, a human transmembrane protein. The authors inserted GFP1-10 and GFP11 into extracellular loops of CD4. They drove expression of the complementary *CD4::GFP* fragments with *him-4*, which is expressed in lateral myocytes, and *ace-4*, which is expressed in dorsal medial myocytes. Muscle cells at the border between dorsal medial and lateral muscle cells fluoresced, suggesting that functional GFP was reconstituted at the junction of *him-4*- and *ace-4*-expressing cells.

PTP-3A is a presynaptic transmembrane protein in *C. elegans*. Neuroligins are usually localized to postsynaptic sites; however, the authors localized the *C. elegans* neuroligin homolog (*nlg-1*) to both pre- and postsynaptic sites. Which transmembrane protein pair with complementary GFP insertions best labels synapses? The authors tested CD4:CD4, PTP-3A:CD4 and NLG-1:NLG-1 pairs. In the ventral nerve cord, AVA command neurons contact VA and DA motor neurons through synapses and gap junctions. The authors drove expression in AVA neurons with the *rig-3* or *flp-18* promoter and drove expression in VA and DA neurons with the *unc-4* promoter. The CD4 pair induced fluorescence throughout the ventral nerve cord. In contrast, PTP-3A:CD4 and NLG-1:NLG-1 pairs induced fluorescence in clusters of distinct puncta,

consistent with the localization of the synaptic vesicle protein RAB-3. These data suggest that tethering GFP fragments to nonsynaptic transmembrane proteins indicates synapses, gap junctions and other cellular contacts, whereas tethering GFP fragments to synaptic transmembrane proteins is important in the specific identification of synapses.

C. elegans lacking *unc-4* did not show GRASP labeling of AVA-to-VA synapses, suggesting that in addition to identifying synaptic partners in wild-type animals, GRASP can also indicate synaptic deficits in mutant animals. Because researchers have developed many *Drosophila* promoter driver lines, the authors suggest that GRASP might be particularly useful in dissecting complex neural circuits in *Drosophila*.

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1. Feinberg, E. H. *et al.* GFP reconstitution across synaptic partners (GRASP) defines cell contacts and synapses in living neurons. *Neuron* **57**, 353–363 (2008). | Article | PubMed | ChemPort |