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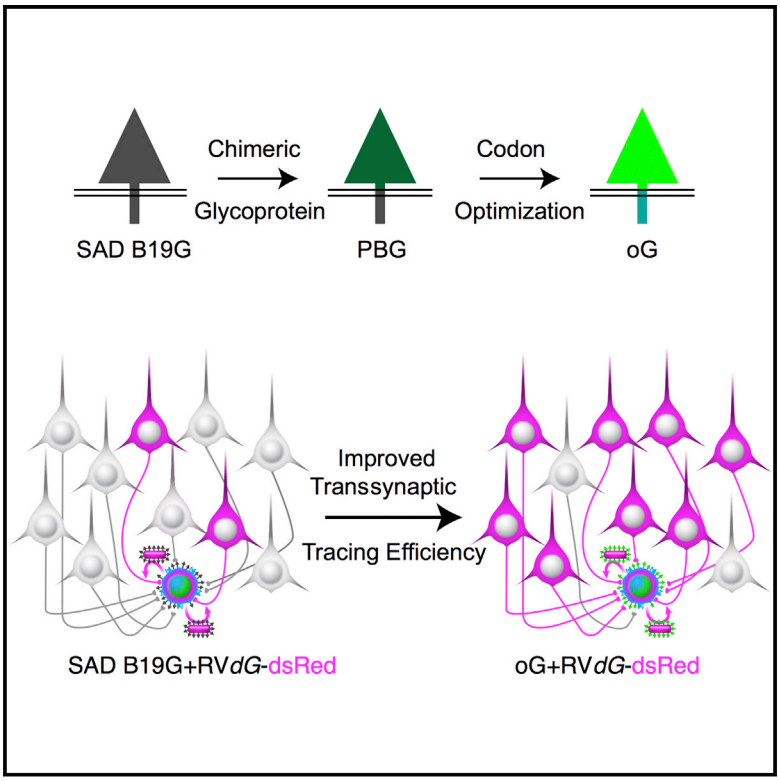
Report

Improved Monosynaptic Neural Circuit Tracing Using Engineered Rabies Virus Glycoproteins

Graphical Abstract



Highlights

- Newly engineered glycoproteins improve monosynaptic rabies tracing
- Optimized glycoprotein (oG) increases tracing efficiency up to 20-fold

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In Brief

Glycoprotein-deleted rabies virus is widely used to trace neural circuits, but it labels only a fraction of all presynaptic neurons. Kim et al. provide a simple method to increase transsynaptic tracing efficiency by adopting the engineered and optimized glycoprotein (oG).

Kim et al., 2016, Cell Reports *15*, 692–699
April 26, 2016
http://dx.doi.org/10.1016/j.celrep.2016.03.067

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Improved Monosynaptic Neural Circuit Tracing Using Engineered Rabies Virus Glycoproteins

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http://dx.doi.org/10.1016/j.celrep.2016.03.067

SUMMARY

Monosynaptic rabies virus tracing is a unique and powerful tool used to identify neurons making direct presynaptic connections onto neurons of interest across the entire nervous system. Current methods utilize complementation of glycoprotein gene-deleted rabies of the SAD B19 strain with its glycoprotein, B19G, to mediate retrograde transsynaptic spread across a single synaptic step. In most conditions, this method labels only a fraction of input neurons and would thus benefit from improved efficiency of transsynaptic spread. Here, we report newly engineered glycoprotein variants to improve transsynaptic efficiency. Among them, oG (optimized glycoprotein) is a codon-optimized version of a chimeric glycoprotein consisting of the transmembrane/cytoplasmic domain of B19G and the extracellular domain of rabies Pasteur virus strain glycoprotein. We demonstrate that oG increases the tracing efficiency for long-distance input neurons up to 20-fold compared to B19G. oG-mediated rabies tracing will therefore allow identification and study of more complete monosynaptic input neural networks.

INTRODUCTION

Since its introduction in 2007, monosynaptic neural circuit tracing using glycoprotein (G)-deleted rabies virus (RV*dG*) has been a powerful and widely adopted tool for the study of neural circuit organization and function (Callaway and Luo, 2015; Wickersham et al., 2007b). Traditionally, the intact rabies virus has been used as a retrograde transsynaptic tracer (Callaway, 2008; Ugolini, 2008). However, since the intact rabies spreads across multiple synapses, it was challenging to identify clear input and output relationships between rabies-labeled neurons. By using RV*dG* and pseudotyping it with EnvA, it is possible to target rabies virus infection to specific starter cell types of interest or even single neurons and to transsynaptically label only their direct presynaptic inputs throughout the mammalian brain (Wickersham et al., 2007b). With this approach, G, which is essential for the transsynaptic spread of rabies virus, is deleted from the rabies genome and replaced by a transgene such as GFP. Consequently, RV*dG* infection is unable to spread except when G is provided in *trans*. Following *trans*-complementation, RV*dG* can utilize G to assemble functional rabies virus particles (G+RV*dG*) that can spread retrogradely across synapses and infect directly presynaptic neurons. The RV*dG* labels these presynaptic neurons by expressing transgenes of interest, such as GFP, but is unable to spread further due to the absence of G. Due to its ability to spread across only one synaptic step in the retrograde direction, RV*dG* has been used widely to delineate brain-wide monosynaptic connectivity to populations of neurons in both the central and peripheral nervous system (Callaway and Luo, 2015).

One important limitation of current monosynaptic rabies tracing is that only a fraction of presynaptic neurons are labeled (Callaway and Luo, 2015). Several lines of evidence suggest that G is a limiting factor (Callaway and Luo, 2015; Miyamichi et al., 2013); for example, increasing levels of G expression by either using a stronger promoter or using a cassette that expresses only G both result in improved tracing efficiency (Miyamichi et al., 2013; Watabe-Uchida et al., 2012). Therefore, improvements in labeling might be achieved by increasing the efficiency of packaging of G into rabies virus particles, improving the uptake of rabies particles by presynaptic neurons, or increasing levels of G expression in starter neurons. Improved efficiency of transsynaptic spread using G-deleted rabies virus of the CVS-N2C strain is consistent with these possibilities (Reardon et al., 2016). Here, we attempt to optimize all three of these factors. We designed additional rabies glycoproteins and tested their efficiency for *trans*-complementation and labeling of presynaptic neurons. We then codon optimized the best of these glycoproteins to maximize expression levels. This modified rabies glycoprotein, oG, significantly improves the efficiency of long-distance transsynaptic tracing with SAD B19 RV*dG*.

RESULTS

To improve the current monosynaptic rabies virus tracing system, we designed and engineered rabies glycoprotein variants different from the original B19G. Various strains of rabies virus display different degrees of virulence and pathogenicity associated with synaptic spread (Schnell et al., 2010). Previous studies showed that retrograde infectivity and transsynaptic spread could be improved in rabies glycoprotein pseudotyped lentivirus or HEP-Flury *dG* rabies system by using G from different strains of rabies (Kato et al., 2011b; Mori and Morimoto, 2014). Because