Mechanisms of neurological dysfunction in *GOSR2* Progressive Myoclonus Epilepsy, a Golgi SNAREopathy

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ABSTRACT

Successive fusion events between transport vesicles and their target membranes mediate trafficking of secreted, membrane- and organelle-localised proteins. During the initial steps of this process, termed the secretory pathway, COPII vesicles bud from the endoplasmic reticulum (ER) and fuse with the cis-Golgi membrane, thus depositing their cargo. This fusion step is driven by a quartet of SNARE proteins that includes the cis-Golgi t-SNARE Membrin, encoded by the *GOSR2* gene. Mis-sense mutations in *GOSR2* result in Progressive Myoclonus Epilepsy (PME), a severe neurological disorder characterized by ataxia, myoclonus and seizures in the absence of significant cognitive impairment. However, given the ubiquitous and essential function of ER-to-Golgi transport, why *GOSR2* mutations cause neurological dysfunction and not lethality or a broader range of developmental defects has remained an enigma. Here we highlight new work that has shed light on this issue and incorporate insights into canonical and non-canonical secretory trafficking pathways in neurons to speculate as to the cellular and molecular mechanisms underlying *GOSR2* PME.

Key words: *GOSR2*, Membrin, Golgi, secretory pathway, Progressive Myoclonus Epilepsy

Abbreviations: SNAP, Soluble NSF-attachment Protein; SNARE, SNAP Receptor; *GOSR2*, Golgi SNAP Receptor complex member 2; PME, Progressive Myoclonus Epilepsy; ER, endoplasmic reticulum; COPII, Coat Protein complex II; ERGIC, ER-Golgi Intermediate Compartment; EPSP, excitatory post-synaptic potential; AMPA, Alpha-Amino-3-Hydroxy-5-Methyl-4-Isoxazole Propionic Acid; TRP, Transient Receptor Potential; CSP, Cysteine String Protein; ANCL, adult-onset neuronal ceroid lipofuscinosis; BMP, Bone Morphogenetic Protein; DIV, days in vitro.

INTRODUCTION

The Progressive Myoclonus Epilepsies (PMEs) are a heterogeneous group of neurological disorders characterised by progressively worsening myoclonus (involuntary muscle jerks), ataxia (uncoordinated movement) and seizures. PMEs can be subdivided into those that are also associated with neurodegeneration and dementia (such as neuronal ceroid lipofuscinoses), and those where cognitive decline is absent or subtle (the prototypical example of which is Unverricht-Lundborg disease) (Minassian, 2014; Ramachandran et al., 2009). PME associated with mutations in *GOSR2* (*GOSR2*-PME) is an example of the later subtype, in which cognitive function is largely preserved (Corbett et al., 2011). Patient symptoms in *GOSR2*-PME emerge early in development, beginning with ataxia with an average age of onset of 2 years old (Boisse Lomax et al., 2013). Action myoclonus of a cortical origin then develops (average onset, 6-7 years old), followed by tonic-clonic seizures (average onset, 13-14 years old), with patient symptoms worsening throughout life (Boisse Lomax et al., 2013; van Egmond et al., 2014). Other notable clinical features include an absence of deep tendon reflexes, scoliosis, syndactyly and partial motor neuron denervation (Boisse Lomax et al., 2013; Corbett et al., 2011; van Egmond et al., 2014), though these are not fully penetrant. Early mortality prior to the age of 30 is common (Boisse Lomax et al., 2013).

The above description illustrates the devastating nature of *GOSR2*-PME. Yet given the fundamental cellular function of Membrin (the protein encoded by the *GOSR2* gene), it is in fact surprising that *GOSR2* mutations result in a predominantly neurological disease rather than system-wide dysfunction and lethality. This is because Membrin plays a critical and conserved cellular role in Eukaryotic species as diverse as yeast, fruit flies, and mammals (Hay et al., 1997; Praschberger et al., 2017; Shim et al., 1991). During the early stages of the secretory pathway, secreted, membrane, lysosomal and endosomal proteins are inserted into the ER lumen or membrane and trafficked from the ER in COPII-coated vesicles, which bud from the

ER and fuse firstly with the ER-Golgi Intermediate Compartment (ERGIC) and subsequently the cis-Golgi (Appenzeller-Herzog and Hauri, 2006; Palade, 1975) (Figure 1). Lipids destined to be incorporated into the plasma membrane (plasmalemma) or membrane-enclosed organelles are similarly trafficked through this route (Pfenninger, 2009). Membrin and its yeast ortholog Bos1 are SNARE (SNAp REceptor) proteins essential for COPII vesicle fusion with the cis-Golgi (Hay et al., 1997; Hay et al., 1998; Shim et al., 1991) (Figure 1). Furthermore, Eukaryote genomes do not harbour redundant Membrin/Bos1 paralogs. Given the requirement of protein and membrane trafficking for cellular viability, it is thus unsurprising that null mutations in Membrin/Bos1 cause lethality in fruit flies and mice (Ghabrial et al., 2011; Meehan et al., 2017; Praschberger et al., 2017), and inhibit mitotic growth in yeast (Shim et al., 1991). Yet despite the essential role of *GOSR2*/Membrin, *GOSR2*-PME mutations are compatible with largely normal human development and cognition, notwithstanding numerous other neurological manifestations. Collectively, these observations raise important questions regarding how *GOSR2*-PME mutations predominantly impact neuronal function and/or development while leaving other organ systems comparatively intact.

In this review we address this apparent paradox. We describe recent work from our group and others examining how *GOSR2*-PME mutations alter both vesicular fusion and neuronal development and excitability. We then expand beyond these proximal causes to hypothesise as to the downstream molecular mechanisms that might underlie multi-faceted neurological dysfunction in *GOSR2*-PME.

STRUCTURAL AND FUNCTIONAL CONSEQUENCES OF *GOSR2*/MEMBRIN MUTATIONS

Membrin is a single-pass transmembrane protein predominantly localised to the cis-Golgi that harbours a critical SNARE domain comprising residues 129-182 (Kloepper et al., 2007; Volchuk et al., 2004). At the cis-Golgi membrane, Membrin acts as a target-SNARE (t-SNARE) in concert with Sec22p and Syntaxin-5 (Hay et al., 1997; Hay et al., 1998). The Membrin/Sec22p/Syntaxin-5 t-SNAREs form a fusogenic complex with the vesicle-SNARE (v-SNARE) Bet1, which is localised to COPII vesicle membranes (Hay et al., 1997; Hay et al., 1998) (Figure 1). Corresponding yeast orthologs perform analogous functions (Newman et al., 1992; Newman et al., 1990; Shim et al., 1991).

Fusion between COPII vesicle and cis-Golgi membranes is driven by interactions between the SNARE domains of Bet1 and the Membrin/Sec22p/Syntaxin-5 t-SNAREs. Fifteen mostly hydrophobic residues (termed layers -7 to +8) are located every 3-4 residues along the SNARE domain, with the central layer 0 composed of a positively charged arginine or glutamine residue (Sutton et al., 1998) (Figure 2). When in close proximity, SNARE domains 'zipper up' along these interacting layers (Sutton et al., 1998). The formation of this trans-SNARE complex occurs via an initial slow N-terminal assembly followed by rapid C-terminal zippering, with this last step imparting the driving force required for membrane fusion (Gao et al., 2012; Zorman et al., 2014).

Interestingly, the two *GOSR2* mutations associated with PME both alter residues in the Membrin SNARE domain (Corbett et al., 2011; Praschberger et al., 2015) (Figure 2). All bar one *GOSR2*-PME patient studied to date has been homozygous for a mis-sense mutation resulting in a glycine to tryptophan substitution (G144W) in layer -3 of the SNARE domain (Boisse Lomax et al., 2013; Corbett et al., 2011). In addition, a compound heterozygote patient harbouring the G144W allele in trans with a deletion of the codon encoding one of two consecutive lysines between

layers +2 and +3 of the SNARE domain (ΔK163/164) was also recently identified (Praschberger et al., 2015). This patient exhibited a milder disease course than previously reported G144W homozygotes, and at 61 years old was the oldest *GOSR2*-PME patient identified to date (Praschberger et al., 2015).

Using yeast orthologs to model the cis-Golgi SNARE complex, we recently investigated the effect of these mutations on SNARE domain function. In liposome fusion assays where cis-Golgi v- and t-SNAREs are reconstituted into distinct vesicle pools (McNew et al., 2000; Parlati et al., 2000), we observed that both mutations acted as hypomorphic alleles, reducing but not abolishing liposome fusion. Furthermore, the yeast equivalent of the ΔK163/164 mutation (ΔD196-Bos1) acted as a stronger loss of function allele compared to G144W (G176W-Bos1) (Praschberger et al., 2017).

Although appearing somewhat puzzling given the milder phenotype of the patient carrying both mis-sense and deletion *GOSR2* alleles, these results are nonetheless consistent with the position and nature of each mutation within the SNARE domain. The G144W mutation alters an interacting hydrophobic residue within the N-terminal region, which facilitates the initial slow interaction of SNARE domains (Gao et al., 2012). Correspondingly, we found that simply pre-incubating the vesicle populations overnight at 4°C was sufficient to restore fusogenic competence to G176W-Bos1-containing vesicles, presumably by allowing sufficient time for N-terminal SNARE interactions to occur (Praschberger et al., 2017). In contrast, the ΔK163/164 deletion causes a positional shift of several key hydrophobic residues in the critical C-terminal region of the SNARE domain, which likely disrupts the efficient assembly of this region that is required to drive membrane fusion (Gao et al., 2012). Consistent with this premise, vesicle pre-incubation at 4°C did not rescue fusogenic competence to ΔD196-Bos1-containing vesicles (Praschberger et al., 2017).

Using fluorescence anisotropy, Völker and colleagues also recently examined the effect of these mutations on interactions between yeast cis-Golgi SNARE proteins (Völker et al., 2017). Focusing on isolated SNARE domains in solution (rather than full-length proteins), they found that the ΔD196 mutation similarly reduced the formation of quaternary SNARE complexes. Surprisingly, SNARE complex formation was enhanced, not reduced, by the G176W mutation. However, wild-type Bos1 but not G176W-Bos1 also underwent substantial self-oligomerization in this assay (Völker et al., 2017). The inhibition of oligomerization by the G176W mutation may result in higher levels of free monomeric (and thus fusogenic) G176W-Bos1 compared to wild-type Bos1, explaining the paradoxical increase in SNARE domain interaction observed for G176W-Bos1. In contrast, our liposome fusion assays utilised full-length, vesicle anchored v- and pre-assembled t-SNAREs. This approach avoided contamination by oligomeric SNARE proteins and allowed us to assess the effect of each mutation in the context of the entire functional SNARE complex (Praschberger et al., 2017).

In vivo, the G147W and ΔK163/164 mutations could also potentially impact the localization and/or stability of the Membrin protein. Our recent work demonstrated that over-expressed Membrin containing either mutation correctly localizes to the cis-Golgi in human fibroblasts and does not appear to be expressed at lower levels compared to a wild-type construct (Praschberger et al., 2017), suggesting a limited effect of either mutation on protein localization and stability. In apparent contrast, Membrin expression in fibroblasts derived from a single patient harbouring the G144W mutation was lower compared to cells from two control patients (Praschberger et al., 2017), yet there was substantial variability in Membrin expression even between control cell lines. Thus, a larger cohort of control and patient tissue is required to determine whether the G144W mutation in particular can

also affect the stability of endogenously expressed Membrin as well as inhibit SNARE domain assembly.

Collectively, the above work suggests that homozygosity for the G144W *GOSR2* allele – the predominant *GOSR2* mutation linked to PME – results in a relatively subtle inhibition of ER-to-Golgi trafficking. Indeed, in our *Drosophila* model expressing the corresponding Membrin mutation (G147W), we found that steady-state levels of a membrane-tagged fluorophore were reduced by ~ 20% in axons of ddaC mechano-sensory neurons compared to a wild-type control (Praschberger et al., 2017). These data are consistent with a mild perturbation of secretory trafficking by G144W Membrin, which may account for the non-lethality of G144W Membrin compared to null alleles of *GOSR2*/*membrin*. When considered alongside the primarily neurological symptoms of *GOSR2*-PME, these results also suggest that the nervous system is particularly vulnerable to disruption of the ER-to-Golgi trafficking relative to other organ systems (Figure 2). To understand why this could be, we next examine the fundamental importance of the secretory pathway for controlling neuronal growth and excitability.

ROLES OF THE SECRETORY PATHWAY IN NEURONS

As in all cells, neuronal trafficking of lipids and proteins from the ER to the Golgi is essential for numerous cell biological processes, and we refer readers to several excellent reviews on the functions of ER-to-Golgi transport and the broader secretory pathway in relation to neuronal function and protein trafficking (Cornejo et al., 2017; Hanus and Ehlers, 2016; Jan and Jan, 2010; Lai and Jan, 2006; Pfenninger, 2009). Below, we briefly describe two roles of the secretory pathway that may be of particular relevance to *GOSR2*-PME.

I. Plasmalemmal expansion

Compared to non-neuronal cells, the plasmalemmal surface area of neurons can be vast (Pfenninger, 2009), extending into the millions of square micrometers for neurons with elongated axons and/or highly elaborated dendritic trees (Bear et al., 2001; Palay and Chan-Palay, 1977). The lipids required to drive plasma membrane growth in neurons are synthesized and transported through the ER and Golgi apparatus within the cell body (Pfenninger and Johnson, 1983). Indeed, the sheer volume of membrane trafficking required for neuronal growth has led to the hypothesis that neurons may be highly sensitive to perturbations in the secretory pathway (Pfenninger, 2009). We propose that *GOSR2*-PME represents a clinically relevant validation of this hypothesis.

Neuronal dendritic growth is particularly reliant on ER-to-Golgi trafficking. In cultured hippocampal neurons, the somatic Golgi apparatus is polarised and oriented towards the longest dendrite, preferentially directing cargo to this growing membrane domain (Horton et al., 2005). Consistent with this arrangement, pharmacological and genetic inhibition of post-Golgi and ER-to-Golgi trafficking dramatically suppresses dendritic growth of hippocampal neurons (Horton et al., 2005; Ye et al., 2007). Surprisingly, although axonal growth requires membrane trafficking through the Golgi (Jareb and Banker, 1997), growing axons appear more resilient in the face of reduced secretory trafficking compared to dendrites. For example, knockdown of Sar1 (a GTPase involved COPII vesicle budding from the ER (Nakano and Muramatsu, 1989)) in 2 DIV hippocampal neurons reduces dendritic but not axonal growth (Ye et al., 2007). In contrast, reducing Sar1 in cultured hippocampal neurons prior to plating causes a reduction in axonal growth (Aridor and Fish, 2009), suggesting that robust and early inhibition of secretory trafficking is required to impact the growing axon. In *Drosophila* ddaC sensory neurons dendritic growth is also profoundly reduced by mutations in *Sar1*, *Sec23* and *Rab1*, all of which function in COPII vesicle budding or subsequent anchoring to the cis-Golgi membrane (Lee et

al., 2004; Ye et al., 2007). However, axonal growth remains largely preserved (Ye et al., 2007).

Given the importance of Membrin in ER-to-Golgi transport (Hay et al., 1997; Shim et al., 1991), these findings are pertinent to our understanding of *GOSR2*-PME. In our recent work we generated *Drosophila* models harbouring the equivalent of either *GOSR2*-PME mutation and showed that expression of these mutant forms of Membrin strongly reduced dendritic but not axonal growth in ddaC sensory neurons (Praschberger et al., 2017). Consistent with our in vitro liposome fusion assays, the *Drosophila* equivalent of the ΔK163/164 mutation (ΔK166-Membrin) caused a more profound reduction in dendritic growth compared to the equivalent G144W mutation (G147W-Membrin) (Praschberger et al., 2017). Importantly, these results suggest that partially reducing COPII vesicle fusion can substantially impact dendritic but not axonal growth. This in turn may have significant consequences for synaptic integration and circuit function in the nervous system of *GOSR2*-PME patients.

II. Ion channel trafficking

In neurons, diverse ion channels control the propagation of dendritic graded potentials, axonal action potentials, dendritic back-propagating action potentials, and calcium-activated synaptic vesicle fusion (Lai and Jan, 2006). Thus, tight regulation of ion channel expression within dendrites, axons and the presynaptic domain is critical for robust information transfer within the nervous system. While local translation of transported mRNAs can occur in axons and dendrites (Cornejo et al., 2017; Van Driesche and Martin, 2018), the canonical trafficking route of membrane-localised ion channels involves directed transport via post-Golgi transport vesicles to the dendritic or axonal domains (Cornejo et al., 2017; Hirokawa and Takemura, 2005), necessitating trafficking through the somatic ER and Golgi apparatus.

Due to this requirement, hypomorphic mutations in *GOSR2* might be expected to alter ion channel trafficking in neurons, with a concomitant impact on neuronal

excitability and neurotransmitter release. Consistent with this concept, in our *Drosophila* models of *GOSR2*-PME we observed an increase in seizure susceptibility at the larval stage that correlated with a broadening of excitatory post-synaptic potentials (EPSPs) during repetitive stimulations at the larval neuromuscular junction (Praschberger et al., 2017). Interestingly, this effect on motor neuron excitability was only observed during high-frequency firing; the amplitude and width of single EPSPs was unchanged (Praschberger et al., 2017). In this preparation, severed motor neuron axons are electrically stimulated with a suction electrode (Zhang and Stewart, 2010), bypassing dendritic input. Furthermore, the amplitude and time course of spontaneous miniature EPSPs at the larval NMJ was unchanged in *Drosophila* models of *GOSR2*-PME (Praschberger et al., 2017), implying that the activity of postsynaptic glutamate receptors localized in the muscle are unaffected by *GOSR2*-PME mutations. Collectively, these results suggest that reduced function of *Drosophila* Membrin results in an alteration in motor neuron excitability that modifies action potential waveforms and/or the dynamics of neurotransmitter release during high frequency firing. It is currently unclear which cargos contribute to this phenotype. However, we posit that it is unlikely to be simply due to a net reduction in ion channel trafficking. Instead, we propose that *GOSR2* mutations result in an *imbalance* of ion channel activity due to at least two factors: parallel non-canonical trafficking pathways and differential sensitivity to changes in ion channel expression.

Recent studies have shown that mammalian GluA1 AMPA Receptors (AMPARs) and Kv2.1 potassium channels can be trafficked to dendritic spines and the axon initial segment (AIS) respectively in a Golgi-independent manner (Bowen et al., 2017; Jensen et al., 2017). In the former case, GluA1 synthesised locally in dendrites is trafficked from the dendritic ER to the ERGIC and subsequently to Rab11-positive recycling endosomes, which facilitate insertion of GluA1 to dendritic spines (Bowen et al., 2017). In the latter case, somatodendritic targeting of Kv2.1 channels occurs via the canonical secretory route whereas transport to the AIS is

Golgi-independent (Jensen et al., 2017), with the precise sequence of vesicular fusion events that deliver Kv2.1 to the AIS yet to be defined.

These findings have implications for understanding alterations in neuronal excitability and plasticity in *GOSR2*-PME. Ion channels often undergo post-translational modifications during the secretory pathway. In particular, immature 'core-glycans' (transferred to specific ion channel asparagine residues in the ER) are trimmed and modified by Golgi-localised enzymes such as Mannosidases and Glycosyltransferases to produce mature N-glycosylated proteins (Moremen et al., 2012). In the case of GluA1, core-glycosylated AMPARs that bypass the Golgi exhibit altered activation kinetics and turnover compared to mature glycosylated counterparts (Hanus et al., 2016). Inhibiting canonical ER-to-Golgi trafficking via *GOSR2* mutations may alter the ratio of mature and immature glycosylated forms of GluA1 AMPARs (and potentially other channels), with consequences for synaptic plasticity and dendritic integration. Indeed, a recent study linking compound heterozygous c430G>T (G144W) and c.2T>G mutations in *GOSR2* to muscular dystrophy and seizures (where c.2T>G likely results in aberrant use of a downstream start codon) found reduced glycolyslation of α-dystroglycan in fibroblasts derived from the above patient (Larson et al., 2018), demonstrating that partial loss of Membrin function may alter glycosylation patterns.

For channels such as Kv2.1 that are trafficked to distinct cellular compartments through canonical and non-canonical routes, *GOSR2* mutations may perturb non-canonical pathways to a lesser degree relative to canonical secretory trafficking, resulting in cellular effects on excitability that are compartment-specific. We note that Membrin also localises to the ERGIC, albeit at relatively lower levels compared to the cis-Golgi (Hay et al., 1998; Volchuk et al., 2004). Thus, *GOSR2* mutations may potentially affect non-canonical trafficking routes involving ERGIC. Nonetheless, we would expect the canonical secretory route, which incorporates several Membrin-

dependent steps (ER to ERGIC, ERGIC to cis-Golgi, and intra-Golgi), to be more strongly affected by *GOSR2* mutations compared to Golgi-independent routes.

Advances in epilepsy genetics have also revealed that the human nervous system exhibits a non-uniform sensitivity to perturbations in expression of membrane-associated ion channels and regulators of neurotransmitter release. For example, haploinsufficiency of the sodium channel *SCN1A* and the presynaptic SNARE *STX1B* cause Dravet syndrome and fever-associated epilepsy respectively (Claes et al., 2001; Schubert et al., 2014). In contrast, loss-of-function mutations in *KCNMA1*, *HCN2*, and *KCNJ10* (encoding calcium-activated potassium channel, cyclic nucleotide-gated channels, and voltage-gated potassium channels respectively) are associated with epilepsy only when homozygous; heterozygote carriers are unaffected (Bockenhauer et al., 2009; DiFrancesco et al., 2011; Tabarki et al., 2016). Thus, nervous system function is robust to reductions in the expression of some proteins but not others. Since *GOSR2* mutations are predicted to cause relatively mild trafficking defects, haploinsufficient ion channels and components of the synaptic release machinery may represent particularly relevant classes of cargos to *GOSR2*-PME pathophysiology, deficient trafficking of which may contribute to the generalized epilepsy and cortical myoclonus that are hallmarks of this disease.

FUTURE DIRECTIONS: HYPOTHESES AND SPECULATIONS

To date, our knowledge of the pathophysiological mechanisms underlying *GOSR2*-PME has been hampered by the lack of in vivo models of this disorder. Our recent study, in which we generated *Drosophila* models of both *GOSR2*-PME mutations, represents an initial step towards understanding genotype-phenotype relationships in *GOSR2*-PME and elucidating how partial disruption of the secretory pathway selectively impacts neurons (Praschberger et al., 2017). However, two rounds of whole genome duplication are thought to have occurred in vertebrates following divergence of the insect and vertebrate lineages (Dehal and Boore, 2005). The

duplication and subsequent diversification of vertebrate ion channel paralogs may result in important differences in the complement of Membrin-dependent cargos between *Drosophila* and mammals. Moving forward, it will therefore be critical to perform neuroanatomical and electrophysiological analyses in mammalian models of *GOSR2*-PME and/or iPSC-derived neurons from *GOSR2*-PME patients. Encouragingly, heterozygous loss of *GOSR2* has been recently been shown to cause an abnormal gait phenotype in male mice (Meehan et al., 2017), pointing to a conserved role for *GOSR2* in regulating coordinated locomotion within mammalian species.

Nonetheless, it is interesting to speculate as to which neuronal phenotypes might be shared between *Drosophila* and future mammalian *GOSR2*-PME models. Given the importance of the secretory pathway in controlling dendritic growth in both mammals and flies (Horton et al., 2005; Ye et al., 2007), we predict that *GOSR2* mutations will similarly reduce dendritic growth in mammalian neurons. Due to the increased diversity of ion channels and neuronal subtypes in the mammalian brain, as well as species-specific regulation of ion channel expression, it is more difficult to infer the impact of *GOSR2* mutations on mammalian neuronal excitability from *Drosophila* data. Yet given that many key ion channel subtypes (including sodium, calcium, potassium and TRP channels) are strongly conserved between mammals and *Drosophila* (Wangler et al., 2015), it is tempting to hypothesize that *GOSR2* mutations also disrupt repetitive firing patterns in *GOSR2*-PME patients in a manner that contributes to seizure generation. With these speculations in mind, we suggest two avenues of exploration that may shed particular light on how nervous system dysfunction arises in *GOSR2*-PME.

I. Differential susceptibility of neuronal subtypes

In our initial study involving *Drosophila* models of *GOSR2*-PME (Praschberger et al., 2017), we sought to address why neurons appeared particularly vulnerable to

GOSR2 mutations. While much work remains to fully comprehend this issue, we can now suggest at least two plausible answers: robust secretory trafficking is required to transport the large volume of plasma-lipid membrane required for dendritic growth, and precise spatio-temporal control of the trafficking of ion channels and synaptic proteins is critical to generate appropriate neuronal firing patters. These demands on the secretory pathway are largely unique to excitable cells within the nervous system.

In future studies, a more intriguing question can now be tackled: not 'why neurons?' but instead '*which* neurons?'. Cognition is relatively unimpaired in the majority of *GOSR2*-PME patients (Boisse Lomax et al., 2013; Corbett et al., 2011; van Egmond et al., 2014), suggesting that neuronal function in many regions of the brain remains largely intact in the face of partial perturbation of the secretory pathway. Which cell-types might therefore be most likely to contribute to ataxia, myoclonus and tonic-clonic seizures in *GOSR2*-PME patients? Extrapolating from *Drosophila* models of *GOSR2*-PME (Praschberger et al., 2017), we hypothesise that two populations of neurons may be particularly impacted by reduced secretory trafficking (Figure 3). Firstly, neurons with complex dendritic arborisations, growth of which requires large volumes of membrane trafficking from the ER to the cis-Golgi (Pfenninger, 2009). Secondly, rapid-firing neurons, such as neocortical and Oriens-alveus interneurons (Erisir et al., 1999; Lien and Jonas, 2003). Of note, cerebellar Purkinje cells fulfil both of the above criteria, since their dendritic fields are highly elaborate (Ramón y Cajal, 1995) and they exhibit spontaneous high-frequency action potentials, with a population mean of ~ 39 Hz but in some cases firing at rates of > 80 Hz in cerebellar slices (Hausser and Clark, 1997). Given the well-documented link between Purkinje cell dysfunction and ataxia (Hoxha et al., 2018; Kasumu and Bezprozvanny, 2012), this neuronal subtype is a prime suspect for contributing to motor defects in *GOSR2*-PME. In support of this premise, data from the Allen Brain Atlas suggests that Membrin is highly expressed in the murine cerebellum, as well as the isocortex, olfactory areas and hippocampal formation (Lein et al., 2007). Such

cell-type-selective effects could potentially explain the relatively specific impact of *GOSR2* mutations on brain regions controlling coordinated movement and seizure susceptibility.

II. Critical cargos

Given the sheer number of proteins that pass from the ER to the cis-Golgi in neurons, the challenge of identifying key pathogenic alterations in cargo trafficking in *GOSR2*-PME appears daunting. However, a small-scale analysis of synaptic proteins in *GOSR2*-PME *Drosophila* models suggests that robust reductions in protein expression are far from universal in the nervous system of *GOSR2*-PME patients. We assessed expression of six membrane-associated synaptic proteins at the larval neuromuscular junction in *GOSR2*-PME *Drosophila* models. Surprisingly, only one of these exhibited a significant reduction in steady-state levels (an ~ 30-40% decrease) at this synapse: cysteine-string protein (CSP) (Praschberger et al., 2017). The mammalian ortholog of CSP, cysteine-string protein alpha (CSPα), regulates synaptic proteostasis by controlling the conformation of synaptic proteins such as SNAP-25 (Sharma et al., 2012; Sharma et al., 2011). Furthermore, mutations in *DNAJC5* (encoding CSPα) cause adult-onset neuronal ceroid lipofuscinosis (ANCL), which similarly to *GOSR2*-PME is characterised by ataxia, myoclonus and seizures (Noskova et al., 2011). Thus, in addition to suggesting a potential molecular link between aspects of *GOSR2*-PME and ANCL, these results raise the possibility that the number of neuronal proteins exhibiting large trafficking deficiencies in *GOSR2*-PME may be smaller than at first expected. Future studies examining the expression of ion channels and synaptic proteins in mammalian *GOSR2*-PME models will again be critical for testing this prediction.

CONCLUSIONS

GOSR2-PME is a multifaceted and devastating disorder. Several studies have provided detailed clinical characterisations of the effects of *GOSR2*-PME mutations on movement control, seizure generation, and motor neuron integrity (Boisse Lomax et al., 2013; Corbett et al., 2011; Praschberger et al., 2015; van Egmond et al., 2015; van Egmond et al., 2014). More recently, how *GOSR2*-PME mutations impact Membrin's function as a cis-Golgi SNARE protein has been analysed (Praschberger et al., 2017; Völker et al., 2017). *Drosophila* models of this disease have further revealed widespread effects of *GOSR2* mutations on dendritic growth and information transfer in an insect nervous system (Praschberger et al., 2017). Yet despite these works the precise downstream molecular underpinnings of *GOSR2*-PME remain poorly understood, particularly since only one study to date has investigated the effect of *GOSR2*-PME mutations on neuronal function (Praschberger et al., 2017). Here we have sought to identify key knowledge gaps and generate hypotheses that may guide future investigations of *GOSR2*-PME. These hypotheses will require testing in mammalian models of this disease, complemented by high-throughput systems such as *Drosophila*. While outside the scope of this review, cell adhesion molecules involved in trans-synaptic stability (Ribeiro et al., 2018), and secreted proteins involved in synaptogenesis such as Wnts and BMP ligands (Budnik and Salinas, 2011; Deshpande and Rodal, 2016; Osses and Henriquez, 2014) are also interesting candidates for involvement in *GOSR2*-PME pathophysiology. Future studies may identify critical secretory cargos underlying nervous system dysfunction in *GOSR2*-PME, which in turn may advance our knowledge of how ataxia, myoclonus and seizures arise in other neurological diseases. *GOSR2*-PME thus highlights the on-going clinical relevance of the study of secretory trafficking and SNARE function in neurons.

FIGURE LEGENDS

Fig. 1. The role of Membrin in the secretory pathway. The early stages of secretory trafficking in a Eukaryotic cell are depicted. For simplicity, the ER-Golgi intermediate compartment (ERGIC) is not shown. Anterograde transport of cargo from the endoplasmic reticulum (ER) to the Golgi is mediated by COPII vesicles that bud from the ER and fuse with the *cis*-face of the Golgi stack. This fusion step is mediated by the v-SNARE Bet1 localized to the COPII vesicle membrane (magenta) and the t-SNAREs Membrin, Sec22b and Syntaxin-5 in the *cis*-Golgi membrane (green). Membrin expression is maximally enriched on the *cis*-face of the Golgi and declines from the *cis*- to the *trans*-Golgi (Volchuk et al., 2004).

Fig. 2. Location and functional impact of mutations in the Membrin SNARE domain linked to *GOSR2*-PME. Top: schematic illustrating location of the SNARE domain and transmembrane domain (TMD) within the Membrin amino-acid sequence. Bottom: alignment illustrates amino-acid conservation of the Eukaryotic Membrin/Bos1 SNARE domain between three metazoan (*Homo sapiens*, *Mus musculus* and *Drosophila melanogaster*) and two unicellular fungi (*Saccharomyces pombe* and *Saccharomyces cerevisiae*) spanning ~ 1 billion years of evolutionary divergence. Black shading indicates ≥ 80% amino-acid sequence conservation between the five species; grey shading indicates ≥ 80% functional conservation. Critical hydrophobic layers (-7 to +8) within the SNARE domain are shown in red. Locations of the G144W and K163/164 mutations linked to *GOSR2*-PME are indicated (Boisse Lomax et al., 2013; Corbett et al., 2011; Praschberger et al., 2015). Functional effects of each mutation, based on liposome fusion assays using *S. cerevisiae* Bos1, and likely overall impact on the secretory pathway, are summarized (Praschberger et al., 2017). Neurons may be selectively impacted by *GOSR2*-PME mutations due to their stringent requirement for robust and tightly regulated secretory trafficking.

Fig 3. A hypothetical model of the impact of *GOSR2*-PME mutations on distinct neuronal subtypes. We predict that due to their comparatively high secretory demands, neurons with elaborate dendritic fields may be particularly sensitive to mild disruption of the secretory pathway. Extrapolating from *Drosophila* models, we also predict that information transfer from rapid-firing neurons may be preferentially perturbed by *GOSR2*-PME mutations due to trafficking defects that disrupt the complex balance of ion channels required to maintain burst firing.

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Figure 1

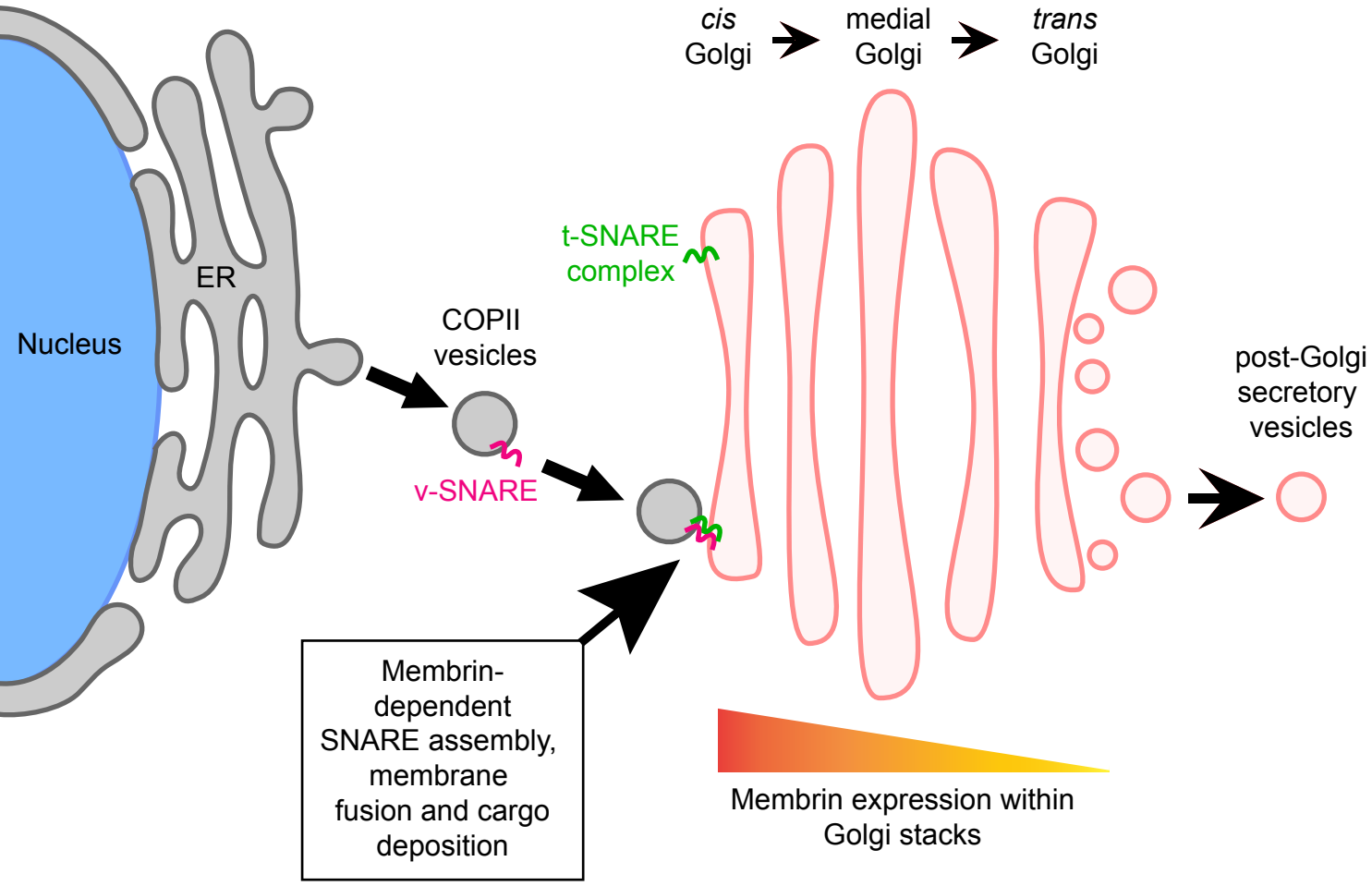

cis Golgi
medial Golgi
trans Golgi
t-SNARE complex
ER
Nucleus
COPII vesicles
v-SNARE
post-Golgi secretory vesicles
Membrin-dependent SNARE assembly, membrane fusion and cargo deposition
Membrin expression within Golgi stacks

Figure 2

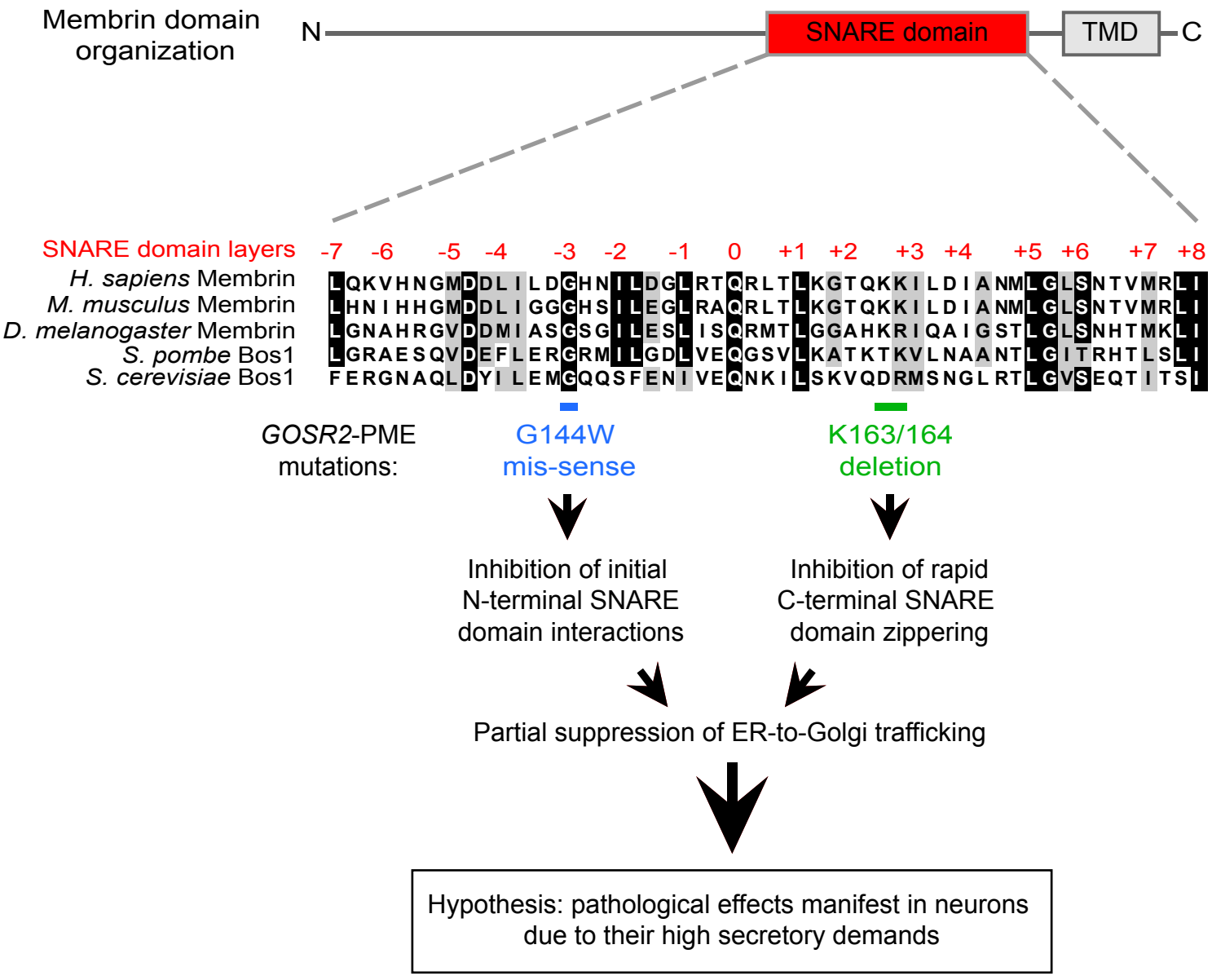
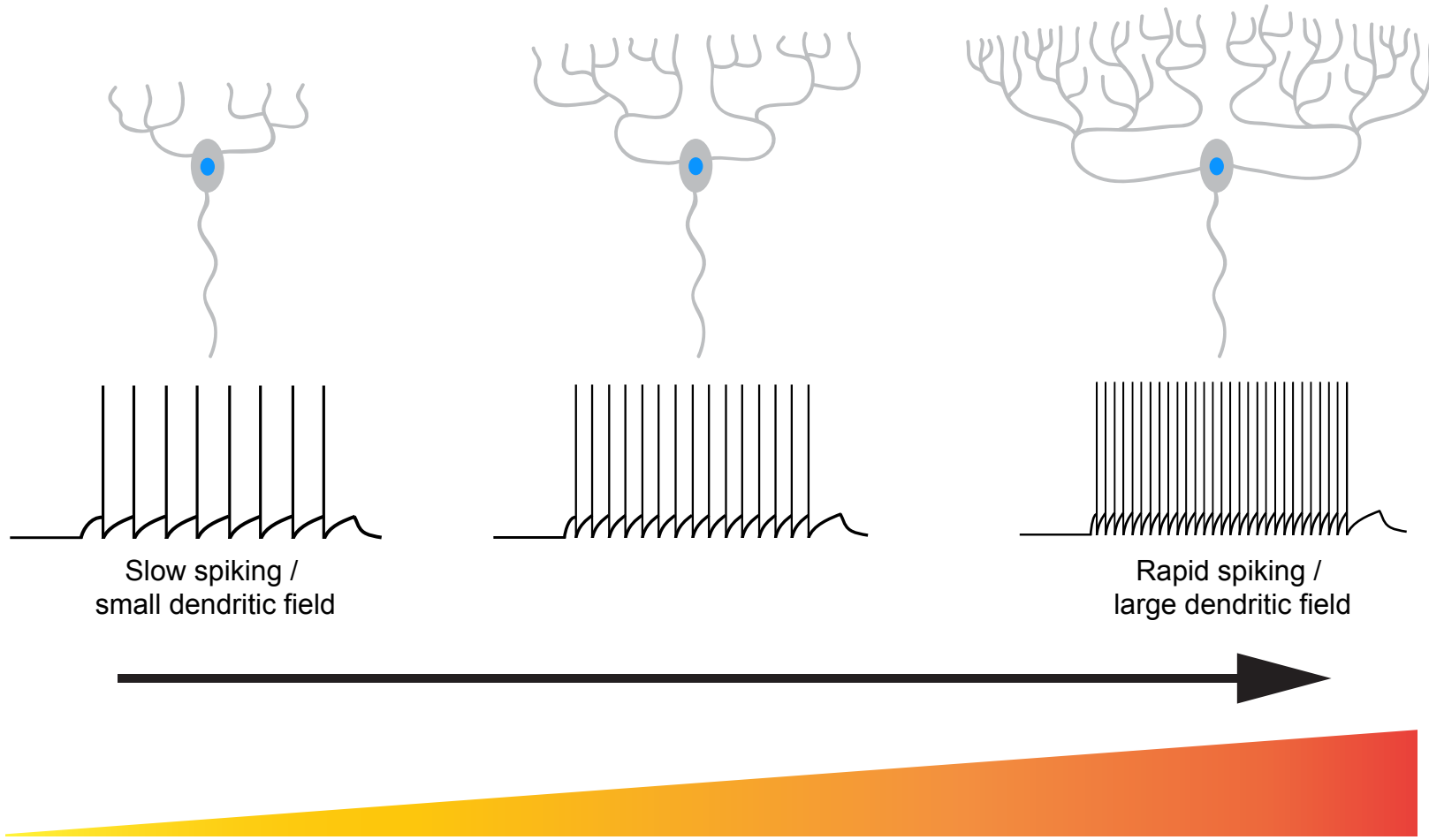

Membrin domain organization
N
SNARE domain
TMD
C
SNARE domain layers
-7 -6 -5 -4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8
H. sapiens Membrin
M. musculus Membrin
D. melanogaster Membrin
S. pombe Bos1
S. cerevisiae Bos1
GOSR2-PME mutations:
G144W mis-sense
K163/164 deletion
Inhibition of initial N-terminal SNARE domain interactions
Inhibition of rapid C-terminal SNARE domain zippering
Partial suppression of ER-to-Golgi trafficking
Hypothesis: pathological effects manifest in neurons due to their high secretory demands

Figure 3


Slow spiking /
small dendritic field
Rapid spiking /
large dendritic field
Relative impact of *GOSR2*-PME mutations