● **PERSPECTIVE**

# Mitochondrial malfunction in vanishing white matter disease: a disease of the cytosolic translation machinery

**Vanishing white matter (VWM) disease – a disease of the cytosolic translation machinery:** VWM is a recessive genetic neurodegenerative disease caused by mutations in any of the five genes encoding the subunits of translation initiation factor 2B (eIF2B) (Leegwater et al., 2001; OMIM 306896). This cure-less disease is rare (rough estimation would be around 1:100,000), but the actual prevalence is thought to be limited by its challenging diagnosis. The disease is characterized by a progressive loss of brain white matter in both hemispheres, causing impairments of neurological functions. Progressive deterioration occurs upon exposure to various physiological stressors, followed eventually by death (Elroy-Stein and Schiffmann, 2015). VWM is in fact a disease of the cytosolic translational machinery. As such, it illuminates the dependence of glial cells on a no-less-than-perfect translational control. The imperfection due to eIF2B mutations is the sum effects of unbalanced global and specific protein synthesis owing to issues of quantities and coordination, with multiple appearances depending on cellular scenarios. A recent study (Raini et al., 2017) provides evidence that homozygous eIF2B5 R132H mutation in mouse (corresponding to R136H in human) leads to impaired oxidative phosphorylation. This cellular phenotype results from a lack of tight coordination between the cytosolic and mitochondrial translation machineries that synthesize the 83 and 13 electron transfer chain (ETC) subunits, respectively. The abnormal ETC composition, confirmed by unbiased mass spectrometry data and biochemical assays (Gat-Viks et al., 2015; Raini et al., 2017), is one of the deleterious effects of imperfect regulation of gene expression due to partial-loss-of-function mutation in eIF2B.

**The vulnerability of different cell types to partial-loss-of-function mutations in eIF2B:** eIF2B is an essential factor required for translation of all mRNAs into proteins within each and every cell type. Due to its unique function (see below), it serves as a master regulator of protein synthesis. Obviously, severe mutations that eliminate eIF2B activity are lethal. However, milder mutations that lead to a tolerated decrease in eIF2B activity are associated with diseased cellular phenotype. The vulnerability of each cell type to eIF2B mutations depends on its functional expertise.

The rate of global protein synthesis is dynamic and varies in response to cellular needs. While reduced eIF2B activity may be adequate to upkeep the required translation rate under basal conditions, it may not be sufficient to support an acute demand for translation upregulation within a short time frame in eIF2B-mutant cells. Since speedy induction of protein synthesis is at risk, cellular tasks that rely on massive protein synthesis within a short time frame are likely to fail upon eIF2B partial-loss-of-function. A relevant example is the response of astrocytes to activation signals that induce

transcription, translation and secretion of growth factors, cytokines and chemokines. Oligodendrocytes experience similar burden during their differentiation from oligodendrocytes precursor cells to mature myelination oligodendrocytes, when they synthesize and assemble large quantities of proteins and lipids to generate myelin sheaths. Glia cells, therefore, are highly sensitive to eIF2B mutations. In contrast, secretory cells equipped with storage vesicles escape the hassle because they synthesize and store their load under normal conditions while the physiological signal elicits merely the secretion step. In agreement with the above, no significant effect of a VWM-related mutation in eIF2B5 was observed on global protein synthesis rate under normal condition in primary fibroblasts, astrocytes and microglia isolated from eIF2B5$^{R132H/R132H}$ mice (Geva et al., 2010; Cabilly et al., 2012). Similarly, it did not affect the total proteome size of whole brains and primary fibroblasts (Gat-Viks et al., 2015; Raini et al., 2017). However, the phenotype of eIF2B5 R132H mutation became apparent in pure primary cultures of astrocytes and microglia upon strong stimulation of gene expression elicited by exposure to lipopolysacharide (Cabilly et al., 2012). This is not surprising, given that both cell types are designed to synthesize and secrete cytokines and chemokines within a short time window in response to a stimulatory signal which turn their status from 'resting' to 'activated'.

**The connection of eIF2B to integrated stress response (ISR) and the differential effect of eIF2B mutation on translation rate of individual mRNAs:** eIF2B is the guanine exchange factor (GEF) of eIF2. By recycling the inactive eIF2-GDP back to active eIF2-GTP, eIF2B enables the formation of eIF2-GTP-Met-tRNA$_i^{Met}$ ternary complex (TC). The cellular concentration of TC dictates the rate of translation initiation. TCs are delivered to 40S ribosomal subunit to generate 43S pre-initiation complexes which enable start codon recognition at each round of translation initiation step. Importantly, the activity of eIF2B is regulated in response to cellular needs. Down-regulation and up-regulation of eIF2B enzymatic activity lead to changes in cellular TC levels, thereby affecting both global and specific mRNA translation. Under stress conditions, one of the four eIF2α kinases (general control nonderepressible 2 (GCN2), proline-rich receptor-like protein kinase (PERK), protein kinase R (PKR) or heme regulated inhibitor (HRI), depending on the stress) is activated and phosphorylates the alpha subunit of eIF2, thereby turning it from a substrate of eIF2B to a competitive inhibitor (Wek et al., 2006). The low eIF2B/eIF2 molar ratio guarantees that even modest eIF2α phosphorylation level will result in an inhibitory effect on eIF2B activity, leading to decreased concentration of 43S pre-initiation complexes. The subsequent differential effect on the translation rate of multiple mRNAs marks the beginning of an expression program termed ISR which also includes feedback dephosphorylation of eIF2α. As predicted, partial-loss-of-function mutations in eIF2B genes are associated with hyper sensitivity to physiological stress (Kantor et al., 2005). This is explained by the fact that eIF2B is constitutively less active, even prior to the stress-induced increase in eIF2α phosphorylation. It is assumed that eIF2B mutations interfere with the tight coordination of the ISR gene expression program. The hyper sensitivity of eIF2B-mutant cells to physiological stress provides an additional reason for the high vulnerabili-
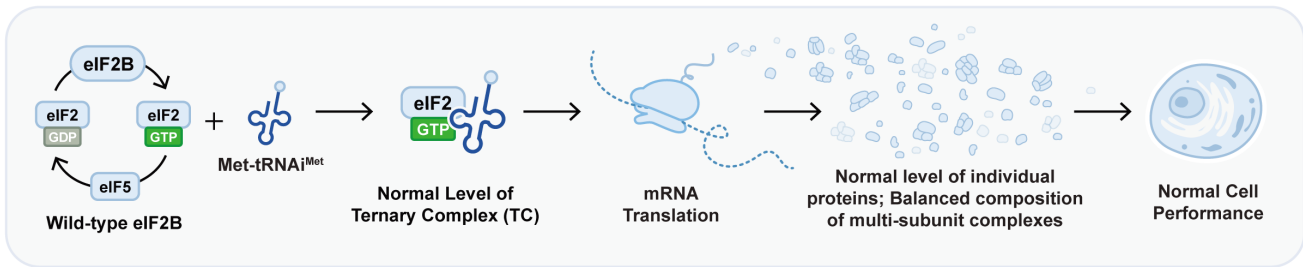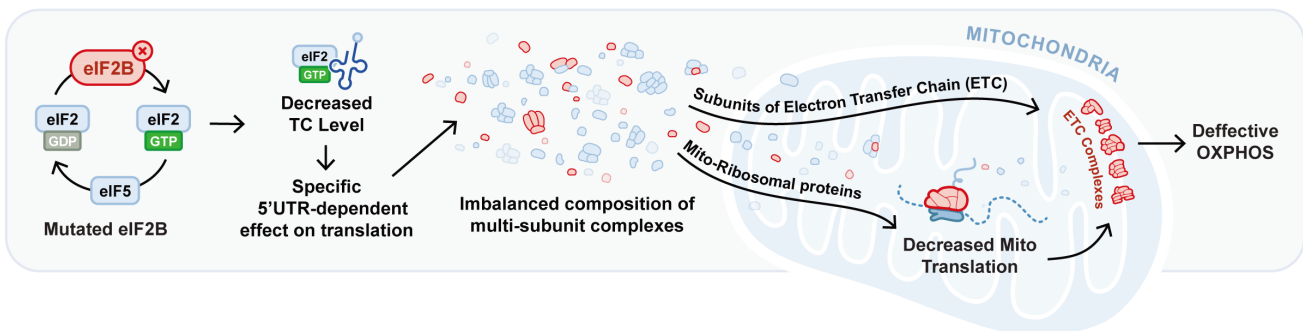
**Figure 1 The effect of translation initiation factor 2B (eIF2B) partial-loss-of-function mutation on cellular phenotype.**
(A) Wild-type eIF2B under normal conditions recycles eIF2-GDP to eIF2-GTP, allowing it to bind charged initiator tRNA to form adequate level of ternary complex (TC). The resulting translation rates facilitate standard abundance of individual proteins and balanced composition of multi-subunit protein complexes, supporting normal cell performance. (B) The lesser enzymatic activity of eIF2B due to loss-of-function mutation results in decreased TC level thereby causing lower concentration of 43S pre-initiation complexes. The consequent differential effect on translation of individual mRNAs (depending on their 5′-untranslated regions (5'UTR)), leads to sub-optimal abundance of individual proteins, thereby causing imbalanced composition of multi-subunit complexes such as electron transfer chain (ETC) and mitochondrial ribosomes. The decreased mitochondrial translation takes its toll on the unbalanced stoichiometry of subunits within the ETC and adenosine triphosphate (ATP)-synthase complexes, leading to defective oxidative phosphorylation and ATP production. eIF5: Translation initiation factor 5; OXPHOS: oxidative phosphorylation.

ty of glial cells. This is not surprising, because the tasks of all glial cells involve massive production of glycoproteins in the ER, rendering them highly dependent on accurate ER-stress response which obviously requires faultless ISR.

ISR gene expression program is primarily based on the differential translation of individual mRNAs depending on their 5′-untranslated regions (5′UTR). Specifically, while a decline in the concentration of 43S pre-initiation complexes leads to reduced global translation, it leads (counter intuitively) to enhanced translation of mRNAs harboring regulatory sequences within their 5'UTR (Young and Wek, 2016). Short upstream open reading frames (uORFs) and internal regulatory entry sites (IRES) differentially regulate gene-specific translation in ISR. Key features of uORFs serve to optimize translational control that is essential for regulation of cell fate in response to physiological and environmental stresses. These features guarantee accurate and timely expression of proteins to alleviate the stress and resume global protein synthesis, or alternatively to promote pro-apoptotic signaling cascade. The inborn differential effect of 43S pre-initiation complexes concentration (affected by eIF2B activity) on translation of individual mRNAs, provokes deleterious outcome in cells expressing eIF2B genes with partial-loss-of-function mutations. In agreement with this notion, abnormal increased/decreased abundances of multiple pro-

teins were detected by mass-spectroscopy measurements of total proteomes of whole brains and cultured fibroblasts isolated from eIF2B5$^{R132H/R132H}$ mice. Analysis of the proteome data exposed unbalanced stoichiometry of subunits within large protein complexes. A major influence on the composition of ETC complexes discovered phenotypic outcome related to defective mitochondrial performance and adenosine triphosphate (ATP) production (Raini et al., 2017; **Figure 1**).

**A multilayered effect leads to defective mitochondrial function:** The effect of eIF2B mutation on oxidative phosphorylation (oxphos) is a striking demonstration of the intricacy of cellular circuits. It highlights the importance of the functional link between the cytosolic and mitochondrial translation machineries and also illuminates eIF2B as an important coordinator. To generate mitochondria the cell needs to express ~1,000 nuclear- and 13 mitochondrial-encoded protein gene products. Oxidative respiration relies on the utilization of five multi-subunit protein complexes (ETC complex I–IV and ATP-synthase) composed of total of 96 protein gene products, only 13 of which are encoded by the mitochondrial genome. Synthesis and assembly of these protein complexes require tight synchronization of gene expression from the two genomes, including coordinated work of the two separated translation machineries. While eIF2B role

is restricted to the cytosol, the mitochondrial translation machinery cannot escape its functional imperfection. The linkage is dictated by the fact that all of the mitochondrial translation machinery components, except mitochondrial ribosomal RNA (mt-rRNA) and mitochondrial transfer RNAs (mt-tRNAs), are nuclear-encoded, thus are translated in the cytosol. Specifically, eIF2B is involved in the synthesis of all mitochondrial ribosomal proteins (MRPs), tRNA synthetases and translation regulatory factors. Altogether, eIF2B partial-loss-of-function mutations can be associated with defective mitochondrial function due to multilayered interconnected events, as illustrated in **Figure 1B**: (i) unbalanced abundance of ETC subunits that are translated in the cytosol; (ii) deleterious effect on mitochondrial translation efficacy due to unbalanced abundance of its cytosolic-translated components; (iii) enhanced harmful effect on ETC composition due to the two above mentioned problems, thereby leading to defective oxphos. In agreement with this notion is the total proteome data collected from mouse fibroblasts homozygous for eIF2B5 R132H mutation (Raini et al., 2017). It shows a spectrum of positive and negative fold-change values of components of the mitochondrial translation machinery with significant changes in abundance of several MRPs of the 39S mitochondrial ribosome subunit.

**More about the immense sensitivity of glial cells to eIF2B mutations:** The discovery of defective mitochondrial performance due to partial loss of eIF2B activity was achieved using primary fibroblasts isolated from eIF2B5$^{R132H/R132H}$ mice. An important note is that fibroblasts do not show any sign of disease in VWM patients. Interestingly, further research revealed that 1.5-fold increase in mitochondrial abundance in eIF2B5-mutant fibroblasts fully compensates for their energy requirements, even without any change in their glycolysis rate, explaining the lack of their involvement in VWM disease. In contrast, 2-fold increase in mitochondrial abundance together with increased glycolysis rate were not sufficient to meet the energy requirement of primary astrocytes isolated from eIF2B5$^{R132H/R132H}$ mice, explaining their increased sensitivity to eIF2B mutations and their involvement in the disease (Raini et al., 2017).

The challenging energy metabolism of astrocytes, the gatekeepers of neuronal energy supply, includes excessive rate of oxphos and glycolysis (Bélanger et al., 2011). Likewise, oligodendrocytes depend on mitochondrial functions and increased energy during the course of myelination (Schoenfeld et al., 2010). Hence, lack of available energy takes its toll on glial cells and renders them extremely sensitive to eIF2B mutations, clarifying the pathophysiology and clinical symptoms of VWM disease. The mitochondrial involvement provides potential targets for the development of a therapeutic strategy.

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