Drug repurposing to target proteostasis and prevent neurodegeneration: accelerating translational efforts

This scientific commentary refers to 'Repurposed drugs targeting eIF2α-P-mediated translational repression prevent neurodegeneration in mice', by Halliday *et al.* (doi:10.1093/brain/awx074).

Neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS) and prion-related disorders (PrDs) are characterized by the aggregation and accumulation of specific proteins that target selective brain regions. These diseases, which currently have no effective treatment, are classified as protein-misfolding disorders (PMDs). Although PMDs develop distinct clinical profiles, emerging evidence suggests that changes at the level of proteostasis maintenance are a common molecular feature. A reduction in the buffering capacity of the proteostasis network during ageing is proposed to increase the risk of neurodegeneration by enhancing the accumulation of abnormal protein aggregates. Since almost one-third of the proteome is synthesized at the endoplasmic reticulum (ER), perturbation of ER function could result in pathological conditions. In fact, ER stress is a common feature of most PMDs, as reported in patient-derived brain tissue and most animal models (Hetz and Mollereau, 2014). Hyperactivation of the ER stress sensor, protein kinase RNA-like ER kinase (PERK) is often co-distributed with degenerating neurons (Scheper and Hoozemans, 2015). Genetic and pharmacological manipulation of this signalling pathway in preclinical models suggests a direct contribution to disease pathogenesis through an abnormal reduction in protein synthesis rates, affecting synapse function and neuronal survival. In this issue of *Brain*, Halliday and co-workers develop an original approach to identify new therapeutics to target proteostasis. Using a library of FDA-approved compounds, the authors performed functional screenings, identifying two compounds that re-establish protein synthesis and provide strong neuroprotection *in vivo* (Halliday *et al.*, 2017). Importantly, one of these drugs is already approved for the treatment of depression, providing an excellent translational candidate for the treatment of neurodegenerative diseases.

To alleviate ER stress, cells activate the unfolded protein response (UPR), an integrated signalling pathway that aims to re-establish proteostasis. PERK is a major UPR transducer that signals through phosphorylation of the alpha subunit of the translation initiation factor 2 (eIF2α) at serine 51. This phosphorylation event results in reduced translation, contributing to alleviate the load of misfolded proteins at the ER. Four different kinases directly phosphorylate eIF2α including, in addition to PERK, protein kinase R (PKR), haem-regulated eIF2α kinase (HRI), and general control nonderepressible 2 kinase (GCN2). This signalling pathway is globally referred to as the 'integrated stress response' (ISR) (Pakos-Zebrucka *et al.*, 2016). Phosphorylation of eIF2α also allows the translation of certain mRNAs containing short open reading frames in the 5'-untranslated region, leading to expression of activating transcription factor 4 (ATF4). ATF4 in turn controls the expression of genes involved in redox homeostasis, amino acid metabolism, autophagy, and protein folding. Under chronic ER stress, ATF4 also regulates the expression of proapoptotic factors, including CHOP and GADD34, engaging the canonical mitochondrial apoptotic pathway. In addition, ATF enhances protein synthesis and oxidative stress to trigger cell demise. GADD34 is part of a phosphatase complex that participates in a feedback loop to dephosphorylate eIF2α (Pakos-Zebrucka *et al.*, 2016). In summary, eIF2α operates as a central rheostat to control cell fate under various conditions by integrating information about the intensity and duration of the stress stimuli.

In normal physiology, phosphorylation of eIF2α is considered neuroprotective. However, when stress is chronic, the inhibition of general protein translation could turn into a deleterious event beyond the induction of apoptosis by perturbing synaptic function. In fact, studies in models of Alzheimer's disease and PrDs have demonstrated that sustained phosphorylation of eIF2α due to PERK hyperactivation negatively affects the synthesis of synaptic proteins, impacting brain function and neuronal plasticity (Moreno *et al.*, 2012, 2013; Ma *et al.*, 2013). These biological activities are shared with other kinases of the ISR pathway, as reported in functional studies aimed at manipulating PKR and GCN2 in the context of synaptic plasticity and Alzheimer's disease. Recent drug discovery efforts to target the PERK/eIF2α pathway have identified interesting novel small molecules. GSK2606414 is a selective inhibitor of the kinase domain of PERK and its oral administration provided strong neuroprotection in prion-infected animals and models of frontotemporal dementia expressing mutant tau (Moreno *et al.*, 2013; Radford *et al.*, 2015). Despite its neuroprotective activity, GSK2606414 has serious side effects, provoking pancreatic β-cell toxicity (Moreno *et al.*, 2013; Halliday *et al.*, 2015).

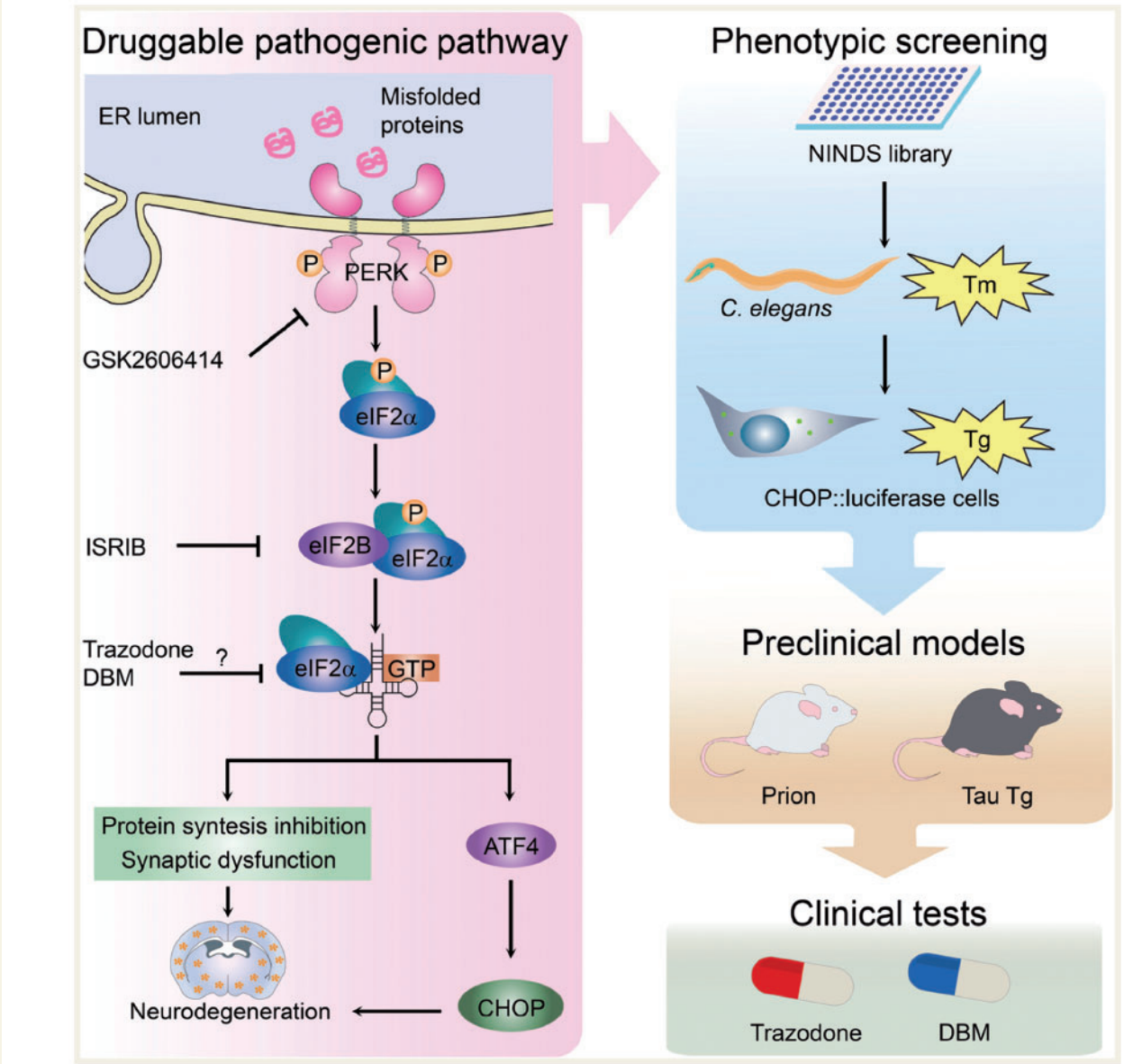

Figure 1 Repurposing drug screening to target proteostasis and prevent neurodegeneration. *Left*: The druggable PERK/eIF2α signalling pathway and the site of action of GSK2606414, ISRIB, trazodone and DBM. *Right*: An approximation of the experimental strategy for a repurposing drug screen based on two stages, phenotypic screening *in vivo* using *C. elegans* and specificity screening using mammalian UPR reporters.

In contrast, ISRIB was identified as a novel small molecule that inhibits ATF4 expression (Sidrauski *et al.*, 2013), and which has a safe toxicological profile in addition to the ability to cross the blood–brain barrier. This molecule blocks the consequences of eIF2α phosphorylation, improving synaptic plasticity in wild-type animals (Sidrauski *et al.*, 2013), as well as protecting against experimental PrD (Halliday *et al.*, 2015). However, this molecule has poor solubility, questioning its translational potential.

Overall, accumulating evidence indicates that strategies to reduce eIF2α phosphorylation may have therapeutic applications in the context of neurodegenerative diseases. In an effort to identify small molecules with clinical value that target eIF2α, Halliday and co-workers developed a phenotypic screen and discovered two compounds from a NINDS small molecule library that reversed translational attenuation triggered by eIF2α phosphorylation (Fig. 1). The screening approach consisted of two consecutive steps (Halliday *et al.*, 2017). First, they tested the ability of 1040 compounds to prevent ER stress-induced developmental delay in *Caenorhabditis elegans* using the glycosylation inhibitor tunicamycin. When nematodes are exposed to

Glossary

ER stress: The cellular condition involving the accumulation of misfolded/unfolded proteins at the ER. ER stress activates UPR stress sensors to adapt to stress or trigger apoptosis of irreversibly damaged cells.

Integrated stress response (ISR): An adaptive pathway in eukaryotic cells that is activated by a range of stress conditions that converge in the phosphorylation of eukaryotic translation initiation factor 2 alpha (eIF2α), which leads to a decrease in global protein synthesis and the induction of selected genes that promote cellular homeostasis.

Proteostasis: A portmanteau of the words protein and homeostasis. Refers to the concept of integrated biological pathways within cells that control the synthesis, folding, trafficking and break down of proteins.

Repurposing drugs: Studying drugs that are already approved to treat one disease or condition to see if they are safe and effective for treating other diseases.

Unfolded protein response (UPR): A signal transduction pathway that is activated in response to an accumulation of unfolded or misfolded proteins in the ER lumen. The UPR mediates the adaptation to protein folding stress or the elimination of non-functional cells by apoptosis.

tunicamycin, the majority of animals do not reach the last larval developmental stage. Using this *in vivo* approach, Halliday *et al.* were able to identify 20 compounds that can overcome the developmental delay induced by ER stress in *C. elegans*. In the second screening, selectivity was tested using UPR-reporter cell lines, restricting the hits to five compounds that reduced CHOP expression under ER stress. Two compounds were then selected for further preclinical studies based on their translational potential, including dibenzoylmethane (DBM) and trazodone. Animals were treated with clinically relevant doses of these two small molecules, which showed impressive therapeutic effects when applied to models of frontotemporal dementia and PrDs. Administration of both drugs provided neuroprotection, reversed memory deficits and delayed disease progression. In addition, trazodone reduced tau phosphorylation. Analysis of the bioavailability and pharmacokinetics of the two lead compounds indicated a remarkable blood–brain barrier penetration and favourable stability. Halliday and coworkers also investigated the site of action of these compounds and determined that both reduced ATF4 levels but not the phosphorylation of eIF2α.

The results presented in the current study introduce the possibility of a major new approach to restoring the functionality of the proteostasis network and potentiating synaptic function and neuronal survival. The two lead compounds represent promising candidates for clinical trials because they are safe and are currently used in patients. However, the general applicability of these findings to the treatment of neurodegenerative diseases should be viewed with caution because many functional studies have also demonstrated a consistent neuroprotective role of the IRS in pathological conditions. For example, compounds that inhibit the eIF2α phosphatase, enhancing translation repression, protect against spinal cord injury, myelin-related disorders, Parkinson's disease, multiple sclerosis and ALS (Hetz and Mollereau, 2014). Thus, fine-tuning eIF2α phosphorylation for therapeutics requires further investigation to understand in more detail possible side effects and determine the optimal administration regimen.

In the past 5 years, the field has witnessed the discovery of common molecular mechanism underlying PMDs, suggesting the potential to translate the findings presented here into therapeutics for a vast spectrum of neurodegenerative diseases associated with ageing. The discovery and design of new drugs takes many years and a great deal of resources; however, this study by Mallucci's group provides a novel framework for drug discovery to accelerate the identification of neuroprotective compounds. Many molecules already approved for human use are available, providing a clear window of opportunity to identify novel future applications. Thus, repurposed drug screenings may help accelerate the discovery of novel disease-modifying agents for many diseases with no current cure.

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