QUALITY CONTROL

Translating translation in Down syndrome

Protein quality control mechanisms may hold the key to treatment of cognitive disability

By **Mark Halliday** and **Giovanna R. Mallucci**

Across the spectrum of neurological disorders, from the developmental to the degenerative, clinical features and progression are influenced not only by disease-specific genetic effects but also by more generic mechanisms. Dysregulated stress responses are emerging as common targets for therapeutic intervention independently of causal genes, offering the tantalizing prospect of new treatments for a swathe of diseases irrespective of specific etiology. The integrated stress response (ISR) is a key player in the control of proteostasis—the balance between protein synthesis and degradation that is essential for cellular health. Dysregulated proteostasis is a common feature of the neuropathological landscape, from fragile X syndrome (*1*) to the neurodegenerative disorders Alzheimer's and Parkinson's diseases (*2*). On page 843 of this issue, Zhu *et al.* (*3*) provide compelling evidence that Down syndrome (DS), the most common genetic cause of intellectual disability, joins the pantheon of neurological disorders in which dysregulated ISR signaling plays a key role.

DS is caused by trisomy of human chromosome 21 (HSA21) and classically features cognitive disabilities, neonatal hypotonia (decreased muscle tone), and craniofacial changes. Cardiac defects and susceptibility to leukemias are less common features of DS. Research on DS etiology has largely focused on determining the functional effects of the extra gene dosage arising from the additional chromosome. A plethora of mouse models of DS have revealed a number of dosage-sensitive genes and genomic regions that contribute to cognitive phenotypes that have formed the basis for candidate therapeutic approaches (*4*).

The ISR controls the rate of protein synthesis initiation (see the figure). A ternary complex, composed of eukaryotic initiation factor 2 (eIF2, comprising α, β, and γ subunits), guanosine triphosphate (GTP, supplied by eIF2B), and a methionine transfer RNA (tRNA), is loaded onto a ribosome for translation to begin at the start codon, AUG, on messenger RNA (mRNA) (*5*). During numerous cellular stresses, it is beneficial to temporarily suspend translation while the stress is resolved to avoid protein misfolding, which is often cytotoxic. This is achieved by phosphorylation of eIF2α, which inhibits the ability of eIF2B to produce the GTP that is essential for ternary complex formation, thereby blocking translation. Four kinases phosphorylate eIF2α and make up the ISR: PKR (protein kinase RNA-activated), a sensor of viral double-stranded RNA and interferon; PERK (PKR-like endoplasmic reticulum kinase), which responds to misfolded proteins in the endoplasmic reticulum and links the ISR to the related unfolded protein response (UPR) (*6*); GCN2 (general control nonderepressible 2), activated by amino acid starvation and HRI (heme-regulated inhibitor), which detects heme deficiency (*7*).

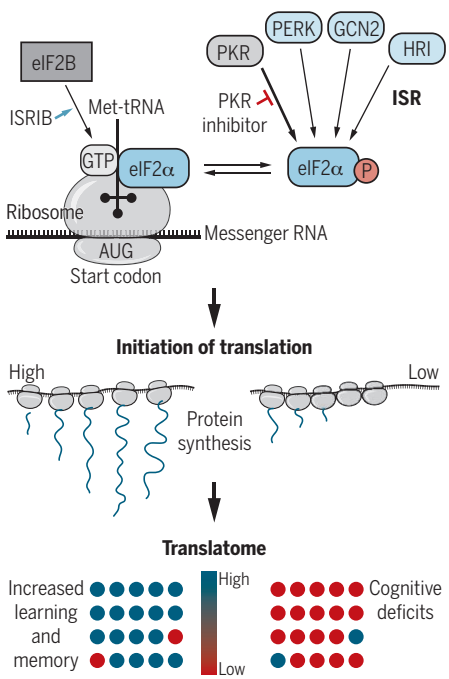
There is mounting evidence that prolonged ISR activation has detrimental effects on cognitive function and neuronal health. Learning and the encoding of long-term memories are dependent on protein synthesis at the synapse, which is controlled by the amount of phosphorylated eIF2α. Inhibiting the ISR either genetically (*8*) or with the ISR inhibitor ISRIB (*9*), or through similarly acting compounds (*10*), improves memory in both wild-type mice and memory-impaired mice. There is also extensive evidence for deregulated ISR in neurodegenerative diseases. Overactive ISR signaling is observed in neurons of patients with Alzheimer's disease, Parkinson's disease, and related disorders, as well as in neurons of mouse models of neurodegeneration (*2*). Reducing eIF2α phosphorylation, either via ISR or UPR modulation, is profoundly neuroprotective in these mice, independent of disease-specific etiology (*2*, *11*).

Zhu *et al.* found reduced translation rates and high amounts of phosphorylated eIF2α, consistent with ISR activation, in the brain tissue of a DS mouse model, in human HSA21 trisomic induced pluripotent stem (iPS) cells, and in postmortem brains from individuals with DS. Intriguingly, they find that it is the PKR axis of the ISR that drives the phosphorylation of eIF2α in DS mice, whereas PERK overactivation is tightly associated with most neurodegenerative disorders (*11*). The DS mice show several behavioral traits thought to be consistent with core cognitive deficits in DS, including reduced ability to learn in several memory tests (*12*). The authors corrected these behaviors by genetic or pharmacological inhibition of ISR signaling, by targeting PKR or the interaction between eIF2α and eIF2B.

How can the reversal of core DS features through ISR modulation be reconciled with

Overactivated stress response in Down syndrome

Trisomy of human chromosome 21 leads to ISR activation through PKR, which phosphorylates eIF2α, reduces global translation rates, and changes the translatome. Approaches that inhibit PKR signaling improve learning and memory deficits in a mouse model of Down syndrome, opening potential new treatments for cognitive disability.



eIF2α, eukaryotic initiation factor 2α; GCN2, general control nonderepressible 2; GTP, guanosine triphosphate; HRI, heme-regulated inhibitor; ISR, integrated stress response; ISRIB, ISR inhibitor; Met-tRNA, methionine transfer RNA; P, phosphorylation; PERK, PKR-like endoplasmic reticulum kinase; PKR, protein kinase RNA-activated.

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evidence from genetic studies that targeting specific pathways and dosage-sensitive genes also rescue some or all of these features? The answer may lie in the translatome of the DS mice that Zhu *et al.* examined. They compared genome-wide transcriptional changes, determined by RNA sequencing, with translational changes, determined by sequencing of polysome-associated (actively translating) mRNA in the brains of mice, and found that numerous genes were transcriptionally and/or translationally dysregulated in DS mice. The translation of more than 80% of downregulated RNAs that were being actively translated was rescued by ablation of the *Pkr* gene in the DS mice, including key proteins involved in learning, synaptic plasticity, and memory storage, which are crucial for cognitive function. Thus, cognitive dysfunction in individuals with DS likely arises, at least in part, from global translatome changes that can be corrected by ISR inhibition.

In the future, a detailed analysis of the altered translatome may contribute to the genetic understanding of DS. Extending translatome analysis to other mouse models of DS and iPS cells from individuals with DS may bring further insights that are also relevant for other features of the syndrome, such as cardiac deficits and susceptibility to leukemias. Why the PKR branch of the ISR, specifically, is activated in DS individuals and mice is unknown and worth exploring. The field of DS research is evolving on many fronts, with drugs targeting specific genes on HSA21 in clinical trials (*13*). It is likely that treatment of DS will ultimately include both generic and specific therapies. The development of ISR inhibitors is a major focus for the treatment of neurodegenerative diseases, including Alzheimer's disease (*2*). The work of Zhu *et al.* raises the possibility that DS may also be amenable to this therapeutic approach, with the prospect of reducing cognitive burden in affected individuals. ■

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DEVELOPMENT

Modeling the early development of a primate embryo

Post-implantation embryos from cynomolgus monkeys are cultured for extended periods

By **Patrick P. L. Tam**

Because mammalian embryos develop inside the uterus after implantation, they are practically inaccessible for direct observation and experimental analysis of the developmental process. To visualize and study the development of post-implantation embryos, it is necessary to develop a technology that maintains the viability and growth of embryos ex vivo in a controlled environment. This is especially the case for nonhuman primate embryos, which are likely to be adopted for modeling early human development. On pages 836 and 837 of this issue, Ma *et al.* (*1*) and Niu *et al.* (*2*), respectively, report in vitro culture methods for cynomolgus monkey embryos and demonstrate their utility for gaining insights into early primate embryo development.

> **"Information generated from the in vitro cynomolgus embryo model will enhance understanding of... early embryogenesis..."**

The laboratory mouse is a road-tested animal model for mammalian development, and its use for investigating the development of pre- to early post-implantation embryos has been substantially enhanced by the ability to conduct experiments on embryos that are grown in culture for various durations between fertilization and early organogenesis (*2*–*5*). However, in view of the disparity of species-specific developmental features between mice and humans, there are reservations about the relevance of translating knowledge from mouse models of embryo development to primates (including humans). The use of human embryos for investigating early post-implantation development is limited by the ethical prerogative of the procurement of and experimentation on human embryos, as well as the technical barrier to sustaining normal growth and development beyond a few days. The ability to grow nonhuman primate embryos to post-implantation development provides a new model of early primate development. The similarity of genomic, anatomical, and physiological attributes between cynomolgus monkeys and humans posits this nonhuman primate embryo as an appropriate animal model for studying human development.

Pre-implantation cynomolgus embryos have been cultured in vitro from the fertilized oocyte, generated by in vitro fertilization (IVF), to the blastocyst at 7 days post-fertilization (dpf) (*1*, *2*, *6*). The availability of cultured IVF blastocysts removes the constraint of sourcing the scarce experimental material (blastocysts or post-implantation embryos) from pregnant animals for further culture and study. However, there has been little success in using present culture methods to support further development of the blastocyst.

The three-dimensional in vitro culture protocols reported by Ma *et al.* and Niu *et al.* have now extended the development of the blastocyst to the equivalent of 19 to 20 dpf (see the figure). By then, the cultured embryos display morphological signs of gastrulation: formation of the primitive streak (where cells move to the new germ layers), emergence of diverse cell types, and acquisition of anterior-posterior polarity. Gastrulation marks a critical developmental milestone of the mammalian embryo, occurring at ~15 dpf in the cynomolgus embryo (*6*), when a diverse range of cell types of the embryonic and extraembryonic tissues are specified and are allocated to the primary germ layers.

Tracking the time course of embryo development in culture, as assessed by morphological landmarks and the appearance of constituent cell types, showed that these embryos have attained the morphogenetic milestones of their in vivo counterparts (*1*, *2*). Overall, the embryos in culture recapitulated the in vivo development of the cynomolgus embryo up to the stage of early gastrulation. However, only a fraction (10 to 22%) of embryos developed normally past the initiation of gastrulation to 20

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