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# Virtual stress plays tricks on cells

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**Optogenetics enables the induction of virtual stress, which separates stress signaling from cellular damage. This provides new insights into the dynamics of the integrated stress response and reveals the mechanisms through which cells form memories of past stress events to guide their response to acute stress.**

New technologies of the digital world allow us to engage in virtual activities. For example, we can simulate stressful situations without putting ourselves in real danger. Besides its common use for entertainment, virtual reality has proven to be helpful in diagnosis and therapy of anxiety and other psychological disorders.[1] Could a similar approach help us to understand how cells react to stressful situations without the need to actually harm them?

Cells in our bodies encounter different forms of stress. Genotoxic stress, for example, threatens the integrity of our genome and is frequently connected to oncogenesis and aging. Cells respond by activating an intricate molecular pathway termed DNA damage response (DDR). While the DDR safeguards our genome, cells also need to ensure that the genetic information is used appropriately to provide the right proteins at the right time. This process is termed proteostasis and comprises protein production, folding, assembly, and degradation as well as the proper localization of proteins. Similar to the DDR, eukaryotic cells have evolved a central mechanism that responds to disturbances of proteostasis, the integrated stress response (ISR).[2] The ISR is triggered by a variety of disturbances ranging from defects in the translation machinery to nutrient deprivation, redox imbalance, and viral infections. Due to its central role in maintaining homeostasis, a malfunctioning ISR is linked to the etiology of neurodegenerative diseases, metabolic diseases such as diabetes, and cancer.

The molecular pathway mediating the ISR is initiated by activation of one of four stress-responsive kinases, which phosphorylate the eukaryotic initiation factor 2$\alpha$ (eIF2$\alpha$). This inhibits the conversion of the GTPase eIF2 to its active, GTP-bound form and thereby downregulates translation of most mRNAs. However, some mRNAs, whose translation is normally restricted by the presence of short open reading frames upstream of the start codon, are now preferentially expressed. These mRNAs include transcription factors (TFs), such as ATF4, which mediate gene expression changes that allow cells to adapt by rebalancing protein production and folding. If the intensity of the stress surpasses the cellular ability to adapt, other TFs such as CHOP induce terminal cell fates including programmed cell death.

Over the last decades, many details of the molecular pathway mediating the ISR have been revealed.[2] However, it is less well understood how the ISR works on a systems level: how do cells encode and decode the intensity and duration of stress to determine appropriate responses? And how do stressors encountered in the past influence the cellular resilience against future stresses?

Analyzing the ISR on a systems level is not trivial. When chemical or physical inducers are used to engage the ISR, they do not only activate the corresponding signaling mechanisms but also cause damage and lead to cascading failures of cellular processes. It is therefore hard to disentangle direct effects of ISR signaling and indirect effects caused by the damage. Another confounding issue is that cellular damage induced by these stressors is not reversible, as repair or replacement of the affected molecules and cellular structures is required.

In this issue of *Cell Systems*, Batjargal et al. report how they devised a way to separate stress inputs from their effects on the cellular machinery and make them reversible to overcome these obstacles. They call it "virtual stress".[3] Instead of inducing ISR kinases via physiological mechanisms, they engineered a version of the stress-induced kinase PKR whose activity can be controlled optogenetically by shining harmless light on cells. Using this approach, they revealed that transient dynamics of the ISR mediates adaptive stress responses by remodeling the proteome and shifting cells to alternate states. In the background, a slow but gradual increase of factors regulating terminal responses prepares the cell for inducing programmed death should the adaptative response turn out to be insufficient to deal with the stress. Both branches of the ISR react proportionally to the activity of the stress-induced kinase. Interestingly, the authors observed hysteresis in their model of the pathway. This suggests that future stress responses depend on the duration and intensity of previous events, generating a stress memory that shapes the response of a cell at a given time.

Optogenetics is a powerful tool that enables precise temporal control over cellular signaling pathways and provides two major advantages[4]: it can be toggled on and off by applying light of defined wavelength, and it is tunable by varying light intensities. This allows scientists to control the temporal and spatial activity of signaling pathways with unrivaled precision and has helped to systematically investigate the underlying signaling logic.[5] Different light receptors are available to optogenetically control signaling through regulating the localization, interactions, or activity of selected proteins. To control the ISR, Wilson and colleagues focused on the kinase PKR, which is activated by double-stranded RNA. The kinase is considered to be part of the innate immune response to viral infections but was shown to be involved in cancer formation and neurodegeneration as well

and plays a role in maintaining organismal homeostasis.[6] It is amenable to optogenetic control due to its activation mechanism, which is based on high-order self-association upon RNA binding. The authors now replaced the modular RNA-binding domains of PKR with variants of *A. thaliana* cryptochrome 2 (Cry2), which act as light-inducible oligomerizers.[7–9] They selected the best-performing variant and termed it "opto-PKR." This light-induced variant of PKR could be activated to similar levels and with similar kinetics as endogenous PKR in response to commonly used ISR activators and induced the expected cellular response.

Using opto-PKR to induce virtual stress, Batjargal et al. dissected the input-output relationship of the ISR and performed genome-wide analysis of gene expression changes at selected time points. This revealed a two-pronged stress response: a subset of regulated genes showed transiently increased or decreased expression levels. These genes mediate adaptation of the cell to the perceived stress. In contrast, the expression of a second set of regulated genes changed slowly but gradually upon virtual stress induction. This subset comprised genes regulating apoptosis, mitosis, and cell adhesion, indicating an involvement in inducing terminal cell fates. It is noteworthy that despite the induction of pro-apoptotic genes, no cell death was observed under the experimental conditions used. Potentially, the ISR needs to be activated for longer or needs to be combined with additional inputs to commit cells to apoptosis.

To gain deeper insights into the dynamics of the signaling pathway, Batjargal et al. turned to a simplified mathematical model. A similar approach has recently been used to demonstrate that the formation of stress granules upon ISR activation by viral infection resembles a stochastic switch.[10] Wilson and colleagues now focused on the role of feedbacks in the network and demonstrated that the model reproduced both receptor-level feedback on opto-PKR itself as well as phosphatase-mediated feedback that acts at the level of phosphorylated eIF2$\alpha$. As the feedback structure of the network generated hysteresis, the authors systematically explored the resulting memory landscape of the stress response by independently varying the duration of stress inputs and recovery periods before querying the cellular response to a uniform challenge. Taking advantage of the high throughput of the optogenetic setup, the authors were able to experimentally validate the predicted relationship between past and future responses.

While the optogenetic uncoupling of ISR signaling and cellular damage provided insights into the signaling logic of the pathway, the next challenge is to further characterize the mechanisms of long-term memory of stress and to understand how stress inputs are processed by the downstream genetic networks to elicit appropriate cell fates. One of the most exciting questions is to understand how cells decide between adaptive and terminal responses. To fully address this question, it will be necessary to investigate how other stress pathways that affect pro- and anti-apoptotic molecules, such as the DDR, are integrated in the memory landscape. In the end, each cell will have its own individual perception of what constitutes a manageable stress. This perception might be modulated by disease and aging, which opens the door for targeting stress pathways therapeutically to alleviate the symptoms caused by malfunctioning memory of past stresses. Similar to the potential of virtual reality for treating psychiatric disorders, virtual stress may therefore provide an efficient way to tackle the detrimental effects of cellular stress.

## DECLARATION OF INTERESTS

The author declares no competing interests.

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