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Life-or-Death Decisions upon Axonal Damage

Francesco Roselli[1] and Pico Caroni[1,*]
[1]Friedrich Miescher Institute, Maulbeerstrasse 66, Basel, Switzerland
*Correspondence: pico.caroni@fmi.ch

In this issue of *Neuron*, Hu et al. (2012) report that upon axonal damage, CHOP and XBP1 unfolded protein response pathways are not recruited equally and have opposite effects on neuronal survival. XBP1 pathway boosting may represent a valuable neuroprotective strategy.

Endoplasmic reticulum (ER) homeostasis, protein synthesis, and protein quality control processes are tightly coordinated events that together ensure a smooth and adequate flow of proteins through cellular compartments, without build-up of misfolded or unfolded proteins. In mammalian cells, disturbances in ER homeostasis trigger three distinct adaptive signaling pathways (Figure 1). First, the accumulation of unfolded proteins activates the ER-resident kinase PERK, whose major substrate is the translation initiation factor eiF2a. Upon phosphorylation of eiF2a, translation is inhibited, thus reducing the load on the folding machinery. In parallel, eiF2a phosphorylation stimulates the translation of a specific subset of mRNAs, including that encoding the transcription factor ATF4. In turn, ATF4 drives the transcription of several critical genes including CHOP, the transcription factor that can trigger the expression of pro-apoptotic genes. A second pathway relies on the bifunctional transmembrane kinase-endonuclease IRE1. Upon detecting unfolded proteins in the ER lumen, IRE1 undergoes multimerization and autophosphorylation, which activates its ribonuclease domain. Active IRE1 is responsible for the unconventional splicing of the mRNA coding for XBP1: when activated, IRE1 ribonuclease removes the intron in XBP1 mRNA, allowing the mRNA to properly code for XBP1, a transcription factor that upregulates ER membrane biosynthesis, ER chaperones, and ER-associated degradation complexes. A third system is based on the cleavage of the transmembrane domain of the transcription factor ATF6. Two proteases cleave and release active ATF6 during its transit through the Golgi apparatus, and the N-terminal ATF6 domain is then free to translocate

to the nucleus where it is a potent inducer of chaperone proteins transcription.

While in yeast the IRE-XBP pathway is responsible for the unfolded protein response (UPR); in mammalian cells all three pathways are involved and have partially overlapping roles (for a review see Walter and Ron, 2011). In nonneuronal cells, triggering a UPR leads to the activation of all three pathways. Of note, a UPR is not induced only as a consequence of acute ER stress, but also as part of a more complex cellular strategy for coping with increased secretory or metabolic requirements, as for example observed in immunoglobulin-secreting plasma cells and in exocrine pancreas (Rutkowski and Hegde, 2010).

The outcome of the UPR is primarily protective, helping cells to survive temporary excesses in protein synthesis requirements or loads of unfolded proteins. However, the UPR triggers apoptosis when restoration of homeostasis is not achieved, and when activation exceeds a threshold in intensity or duration. Accordingly, the same signaling pathways can be anti- or pro-apoptotic depending on the trigger, intensity, and cellular context of UPR activation (Han et al., 2009).

Hu et al. describe a strikingly divergent role of UPR signaling pathways in retinal ganglion cell (RGC) survival and death upon axonal injury. Following three unrelated optic nerve injuring treatments (optic nerve crush, vincristine-induced optic neuropathy, or intraocular hypertension (IOP) mimicking open-angle glaucoma), they identify in retrogradely labeled RGCs a characteristic pattern of UPR activation: a marked and sustained upregulation of CHOP-dependent UPR, along with a lesser and more transient activation of XBP1 splicing (Figure 1). In

parallel, the optic nerve injury protocols produce major losses of RGCs. Notably, optic nerve crush in CHOP KO mice resulted in a major increase in RGC survival, whereas retinal knockdown of XBP1 did not rescue RGC apoptosis. Conversely, AAV-mediated overexpression of spliced (s) XBP1 dramatically preserved RGC survival, with even greater effects detected in a CHOP-KO background. Most notably, the protective effect of sXBP1 overexpression was detected in clinically meaningful conditions, such as when AAV injection was performed subsequent to the experimental establishment of IOP. Unfortunately, these manipulations improved RGC survival but did not promote axonal regeneration. Hu et al. thus identify two at least partially independent UPR pathways triggered upon axonal injury that have opposite roles in determining the fate of RGCs: PERK-CHOP signaling results in RGC apoptosis, whereas XBP1 stimulates cell survival (Figure 1). Notably, in the course of mechanical or pharmacological damage to the optic nerve, modest sXBP1 upregulation appears to be insufficient to counteract CHOP expression, and RGCs undergo extensive apoptosis.

Hu et al.'s results suggest that in neurons a UPR can be an intrinsic response to disturbances in axonal integrity and flow, possibly unrelated to the load of un/misfolded proteins and part of a general response strategy to axotomy (see also Saxena et al., 2009). One possibility is that, in this context, the UPR might be triggered by a specific lesion signal (or the lack of an "integrity signal") generated in the injured axon, to remodel the ER and spur regeneration. The identity and indeed existence of such signals remains to be determined. In principle, the UPR response may be directly triggered by
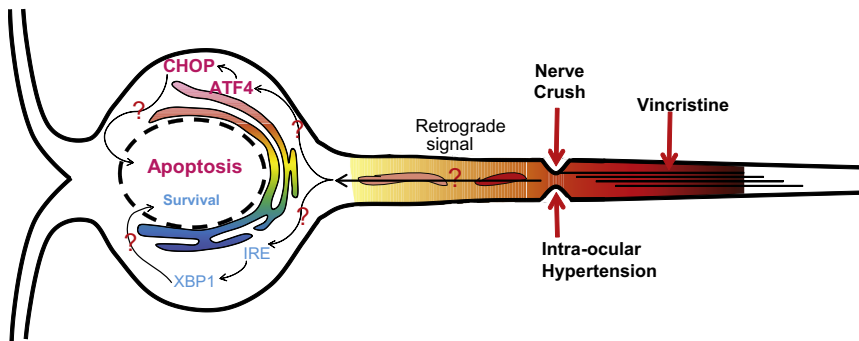
Figure 1. Recruitment of Distinct UPR Pathways Promoting Neuronal Survival and Death upon Axon Damage
Hu et al. (2012) show that upon axonal damage due to optic nerve crush, intraocular vincristine or intraocular hypertension, extensive apoptosis of retinal ganglion cells is the result of the activation of UPR. A robust and sustained activation of the ATF4-CHOP cascade promotes axotomy-induced apoptosis, which is much reduced in CHOP$^{-/-}$ mice. In parallel, a lesser and transient activation of the IRE-XBP1 pathway has protective effects, and AAV-mediated XBP1 overexpression counteracts axotomy-induced RGC apoptosis. The nature of the local and retrograde signaling leading to UPR induction is unknown, as are the effector mechanisms for CHOP-dependent apoptosis and XBP1-mediated neuroprotection.

physical or functional damage to ER tubular membranes in axons, thus providing potential more general scenarios in which axonal dysfunction may produce signaling to the soma to activate repair responses. Whether and how local ER dysfunction in the axon influences neuronal UPR responses remains to be determined. In the specific context of axonal injury, the UPR response appears to mainly have a detrimental outcome. Why the activation of XBP1 splicing is limited, compared to the robust upregulation of CHOP, is unclear; the authors speculate that this may be due to limited amounts of XBP1 mRNA in the axon itself. Alternatively, local splicing may be inefficient, or the retrograde signal may not effectively recruit the IRE-XBP1 pathway. Furthermore, since both IRE1 and PERK are intrinsic ER membrane proteins, activation in specific subdomains of the ER may play a role (Figure 1). Clearly, our understanding of these pathways in neurons, including the ATF6 pathway that was not considered in this context, is still incomplete. Their investigation in future studies might yield valuable information to translate progress in neuronal cell biology into more effective strategies for neuroprotection.

The mechanisms underlying the opposite effects of CHOP and XBP1 pathways on neuronal survival also remain to be investigated. The CHOP cascade appears to have a critical role in UPR-dependent cell death in neurons (Galehdar et al., 2010), and nonneuronal cells (Puthalakath et al., 2007), largely due to the induction of BH3-only pro-apoptotic proteins such as bim and puma. By contrast, the neuroprotective mechanisms set in motions by XBP1 are less clearly understood: the induction of ER chaperons (such as BiP, Grp94, and Grp58) and the stimulation of ER biogenesis (Walter and Ron, 2011) may be important, but further targets of XBP1, possibly including autophagy pathways (see e.g., Hetz et al., 2009) may also have a role.

This study clearly suggests that XBP1 is a valuable neuroprotective target to counteract neuronal losses and blindness upon axonal injuries. But the lessons learned through these axonal damage studies might have implications beyond injury-related cell death and neural repair. Thus, early UPR upregulation is a hallmark of neurodegenerative diseases (for a review, see Saxena and Caroni, 2011). CHOP and XBP1 upregulation has been described in Alzheimer's disease, Parkinson Disease, ALS models (Kikuchi et al., 2006), and photoreceptors expressing mutant rhodopsin (Ryoo et al., 2007)—and may contribute to the pathogenesis of prion diseases (Rane et al., 2008). Notably, XBP1 exerts neuroprotective effects against amyloid-β induced neuronal death in a *Drosophila* model,

although here XBP1 overexpression does not affect ER stress per se, but rather the regulation of cytosolic Ca^{2+} levels upon downregulation of ryanodine receptors (Casas-Tinto et al., 2011). Likewise, XBP1 is upregulated in chemical mouse models of PD, and AAV-mediated XBP1 overexpression in the substantia nigra is neuroprotective in this condition (Sado et al., 2009). As a word of caution, however, XBP1 knockdown can also result in decreased load of misfolded proteins and neuroprotection (Hetz et al., 2009), and CHOP upregulation may not always lead to apoptosis (Halterman et al., 2010). Accordingly, the outcome of IRE-XBP1 and CHOP pathway activation may depend on the identity of the affected neurons, on context, and on the specific triggers that induce the UPR. Clearly, the issues raised by the results of this elegant study have important potential implications for our understanding of how axonal dysfunction influences neuronal function, repair, and death under acute and chronic conditions.

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Curbing Fear by Axonal Oxytocin Release in the Amygdala

Philip Tovote[1] and Andreas Lüthi[1,*]
[1]Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
*Correspondence: andreas.luthi@fmi.ch
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Oxytocin produces anxiolytic effects via the central nucleus of the amygdala but how the peptide reaches its receptors in this region has been unclear. In this issue of Neuron, *Knobloch et al. (2012) demonstrate that evoked oxytocin release from axon terminals within the central amygdala results in attenuation of fear.*

The evolutionarily preserved neuropeptide oxytocin (OT) is perhaps best known for its role as an important hormonal regulator of mammalian reproductive processes such as cervical softening, uterine contraction, and milk ejection. In addition to these peripheral effects, OT is involved in functions of the central nervous system. From enhancing social recognition, pair bonding, and maternal behavior to reducing stress effects and pain sensitivity, central effects of OT have been demonstrated in many mammalian species (Landgraf and Neumann, 2004). OT strengthens pair bonding in monogamous female prairie voles, whereas blocking OT receptors prevents pair bonding. OT can induce maternal behavior in virgin rats whereas rats selectively bred for strong maternal behavior start to neglect their pups when central OT receptors are pharmacologically blocked. In humans, intranasally applied OT attenuates the stress response induced by public speaking, and OT release during breast-feeding lowers stress hormone levels and elevates mood in mothers (Lee et al., 2009). Interestingly, these anxiolytic effects of OT have been associated with reduced neuronal activation in the amygdala, a key brain structure for anxiety and fear (LeDoux, 2000). The central nucleus of the amygdala (CeA), comprising lateral (CeL) and medial (CeM) subdivisions, mediates acquisition and expression of behavioral as well as autonomic fear responses (Maren and Quirk, 2004). Strong OT receptor expression within the CeL has been reported, and in mice, local application of OT in the CeA results in attenuation of conditioned fear responses (Viviani et al., 2011). However, the way by which OT reaches the CeA to affect fear has remained unclear (Neumann, 2007). Neurons of the paraventricular (PVN), supraoptic (SON), and accessory magnocellular (AN) nuclei of the hypothalamus synthesize OT and release it via their axon terminals in the posterior pituitary from which it enters the blood stream. Because OT cannot pass the blood-brain barrier, its effect on CeA function and subsequent fear behavior must be centrally mediated. Axonal projections of hypothalamic OT neurons targeting the limbic system have been reported for olfactory bulb, septum, and hippocampus, but until now, evidence of OT axonal fibers within the amygdala has been limited (Landgraf and Neumann, 2004). Thus, it was proposed that OT, after dendritic release either from unidentified cells in CeA or from magnocellular neurons in the hypothalamus, would passively diffuse within the extracellular matrix to reach distant target regions, including CeA (Neumann, 2007; Ludwig and Leng, 2006).

In general, there are numerous routes through which neuropeptides are released and reach their targets. They can be secreted over the entire cell membrane including soma and dendrites into the extracellular space and ultimately reach receptors by way of diffusion (Ludwig and Leng, 2006). Alternatively, neuropeptides can be coreleased at synapses together with classical neurotransmitters such as GABA or glutamate. Depending on the amount released and because of relatively long half-lives due to slow degradation in the extracellular space, neuropeptides often spill over from synapses to bind extrasynaptic receptors. Passive diffusion along concentration gradients following dendritic release or synaptic spillover presents a mechanism through which neuropeptides, such as OT or vasopressin, without using direct cell-to-cell connections, can modulate the activity of their target cells. However, because these diffusion processes are both slow and undirected, this comes at cost of temporal as well as spatial specificity of neuropetidergic signaling. Focal release of neuropeptides at synaptic sites