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SPOTLIGHT

Disagreement among the three musketeers of the unfolded protein response

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Jipa and Juhász preview results from the lab of Tao Wang (2023. *J. Cell Biol.* https://doi.org/10.1083/jcb.202208147) which show a surprising antagonism between two branches of the unfolded protein response that dictates disease progression in a model of autosomal dominant retinitis pigmentosa.

The unfolded protein response (UPR) responds to the accumulation of misfolded or unfolded proteins in the ER. UPR is essential for maintaining cellular homeostasis, as it allows cells to adapt to stress conditions and maintain ER function. UPR is also involved in a variety of physiological and pathological processes, including differentiation, development, and disease. One such disease is autosomal dominant retinitis pigmentosa (adRP), a genetic disorder that affects the retina: the specialized tissue at the back of the eye that is responsible for detecting light and transmitting visual information to the brain. In adRP, photoreceptor cells of the retina gradually become dysfunctional and die, leading to vision loss and eventually blindness. adRP is caused by mutations in genes affecting the function and maintenance of the retina including rhodopsin (Rho), the visual pigment of rod photoreceptor cells. Pathological mutations of this gene cause ER stress that destabilizes the wild-type protein and leads to degradation of photoreceptor cells (1). This gene has a homologue in *Drosophila melanogaster* called Rh1, which enabled the development of authentic disease models to study the signal transduction pathways involved, such as UPR (2). UPR signaling has three main branches, IRE1, protein kinase RNA-like ER kinase (PERK), and ATF6, but their importance and potential crosstalk during diseases such as adRP has remained an open question. Another issue in adRP is the potential role of autophagy, a lysosomal self-degradation pathway that is usually used by cells to break down pathological proteins and damaged organelles and counteract disease development this way (3).

Zhao et al. now generated a new *Drosophila* model of adRP, which expresses both a pathological variant $Rh1^{P37H}$-GFP and the RFP-tagged wild-type protein (4). Of note, the missense mutant form of Rho-GFP is retained in the ER and causes ER stress in the *Drosophila* eye, while the wild-type protein is still transported to the light-sensing rhabdomeres in photoreceptors. The authors carried out a large-scale chemical mutagenesis screen on this genetic background, which led to the isolation of four mutations that, surprisingly, decrease wild-type Rh1-RFP protein level with a concomitant increase in $Rh1^{P37H}$-GFP. The newly identified mutations affected the genes encoding PERK and one of its effectors, elF2Bα. Importantly, loss of these genes prevented the transport of wild-type Rh1-RFP to rhabdomeres—instead, it was retained in the ER in an immature form together with the mutant protein, causing severe retinal degeneration over time. These genetic interaction data indicated that the PERK signaling branch has an important role in controlling mutant vs. wild-type protein levels in this UPR model. In line with this, the loss of ATF6 or IRE1 had no effect on Rh1 homeostasis. Interestingly, loss of ATF4 (another PERK effector) had no effect either, further confirming the specific involvement of PERK-elF2Bα signaling.

To investigate the molecular mechanism responsible for this phenomenon, the authors carried out RNA sequencing with or without PERK inhibition on an $Rh1^{P37H}$-GFP background. They found upregulation of IRE1/XBP1-induced genes in $perk^{RNAi}$ flies, and knockdown of *ire1* or *xbp1* prevented the loss of wild-type Rh1 seen upon *perk* knockdown. Thus, PERK prevents uncontrolled IRE1 signaling that causes wild-type Rho degradation, which the authors found to occur via selective autophagy of the ER in this model. IRE1 has already been shown to induce autophagy in a neurodegenerative disease setting, but the underlying mechanism remained unknown (5). Proteomic analysis of $Rh1^{P37H}$-*GFP*, $perk^{RNAi}$ flies now detected the upregulation of Ref(2)P/p62 protein, the *Drosophila* ortholog of mammalian p62/SQSTM1, which is a critical cytosolic receptor for selective autophagy of ubiquitinated cargo (6). Further experiments revealed the transcriptional upregulation of *ref(2)P/p62*, along with an ER-associated ubiquitin ligase, and multiple *Atg* genes encoding core proteins necessary for autophagosome formation.

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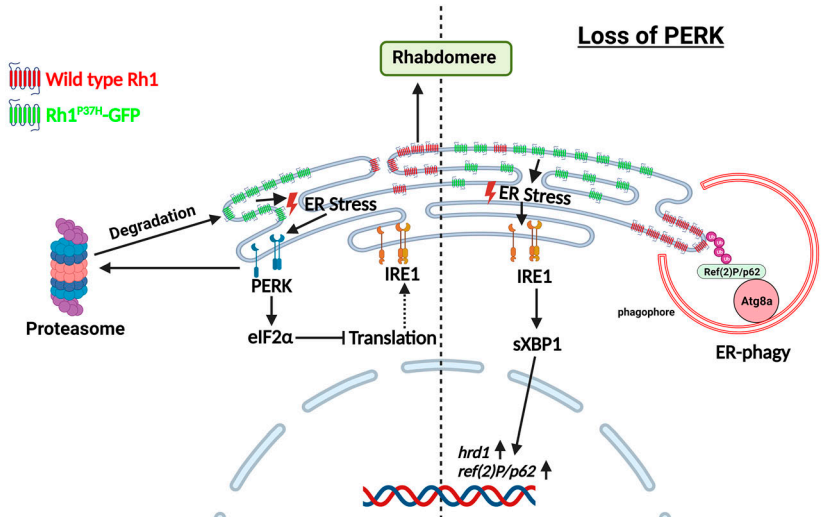

Figure 1. **Model of the cell-protective role of PERK in adRP.** Left side: Photoreceptor cells counteract ER stress caused by mutant Rh1^{P37H}-GFP expression through PERK-dependent proteasomal degradation of the pathogenic protein. At the same time, PERK signaling inhibits IRE1. Right side: Loss of PERK results in accumulation of mutant Rh1, as it is no longer degraded by the proteasome, and IRE1 is activated. This initiates a transcriptional cascade triggering Ref(2)P/p62-dependent ER-phagy for selective autophagic degradation of wild-type Rh1. These changes are responsible for accelerated neurodegeneration seen in adRP model flies with loss of PERK. See text for more details.

Strikingly, IRE1/XBP1-dependent induction of Ref(2)P/p62 was found to mediate the breakdown of wild-type Rh1 protein by selective ER autophagy, while the mutant protein escapes degradation if PERK is lost in this adRP model. This is surprising because PERK is known to induce, rather than inhibit, autophagy in other settings such as *Drosophila* fat cells (7).

To find out how mutant Rh1^{P37H}-GFP protein is degraded, the authors investigated the role of the ubiquitin-proteasome system in this disease model. Interestingly, not only ubiquitinated Rh1^{P37H}-GFP accumulated but there was a general increase in the level of ubiquitin in *perk*^{RNAi} photoreceptors. The strong accumulation of a proteasome activity reporter indicated that loss of PERK inhibits proteasomal degradation in general. All these data support that mutant Rh1^{P37H}-GFP protein is degraded by the proteasome, which requires PERK in this adRP model (Fig. 1).

To validate these new results, the authors turned to a classic *Drosophila* model of adRP: the dominant negative mutant allele *ninaE*^{G69D} that causes age-dependent photoreceptor degeneration (8). In this model, they also observed transcriptional upregulation leading to accumulation of Ref(2)P/p62 protein, along with increased punctate Atg8a indicating autophagy induction, and loss of ER but not mitochondria. Furthermore, Atg8a puncta formation was impaired in *ref*(2)*P*/*p62* mutant flies, indicating that Ref(2)P/p62-dependent selective ER-phagy has an important role in the process. Importantly, overexpression of PERK (but not ATF4) decreased the level of Ref(2)P and prevented the loss of rhabdomeres and photoreceptor cells.

Overall, this article achieved significant progress in understanding adRP disease mechanisms, UPR signaling, and regulation of ER-phagy in a disease setting. Of the "three musketeers" activated by ER stress, PERK is a key guardian in adRP: it not only promotes proteasomal degradation of the pathogenic mutant form of Rho, but it also antagonizes the other signaling branch IRE1, whose activation has detrimental consequences on disease progression. Specifically, uncontrolled IRE1 activity leads to transcriptional upregulation of *ref*(2)*P*/*p62* and other genes that cooperatively induce ER-phagic breakdown of wild-type Rho. This then accelerates loss of vision and neurodegeneration. It is particularly interesting that levels of the selective autophagy receptor Ref(2)P are increased and that this increase is required for selective ER-phagy upon ER stress, because Ref(2)P protein levels are normally decreased during autophagy due to its selective degradation. Most importantly, autophagy is generally considered as a cell-protective mechanism that promotes cell homeostasis and survival (3). Since loss of PERK induces IRE1-dependent selective ER-phagy that leads to degradation of wild-type Rho, whereas overexpression of PERK suppresses selective autophagy and prevents neurodegeneration in this adRP model, it is a clear demonstration of a disease setting in which autophagy is a bad guy, raising the possibility that adRP patients may benefit from lower rather than higher levels of autophagy in their photoreceptor cells.

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