Copper Regulation of the PERK Arm of the Integrated Stress Response

Noah R. Beratan, Sarah E. Bond, Donita C. Brady, PhD. Kelly L. Jordan-Sciutto, PhD

University of Pennsylvania

Abstract

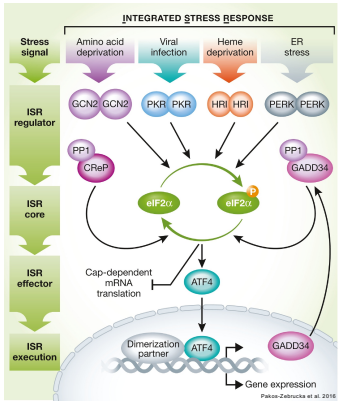
The treatment of many neurocognitive disorders, including HIV-associated neurocognitive disorders (HAND), has remained challenging as the underlying mechanisms of pathogenesis are not fully understood (Akay). However, the phosphorylation of eukaryotic initiation factor 2α (eIF2α) is common amongst many of these disorders (Hetz). Phosphorylation of eIF2α can be mediated by 4 kinases that make up the integrated stress response (ISR), which responds to a variety of stressors including endoplasmic reticulum (ER) stress (Pakos-Zebrucka). Protein kinase R-like ER kinase (PERK) is one of the kinases that activates the ISR and mainly responds to the accumulation of unfolded proteins. When ER stress is sensed, PERK oligomerizes and autophosphorylates to activate, then phosphorylates eIF2α, setting off a signaling cascade that causes global translation attenuation with selective upregulation of enzymes that help fold proteins and resolve the stress (Walter). However, chronic activation can lead to cell death (Urra). Recent studies have proven that copper can act as a novel regulator of kinase activity (Brady, Tsan). Here we investigate the dependence of PERK activity on copper availability in cells.

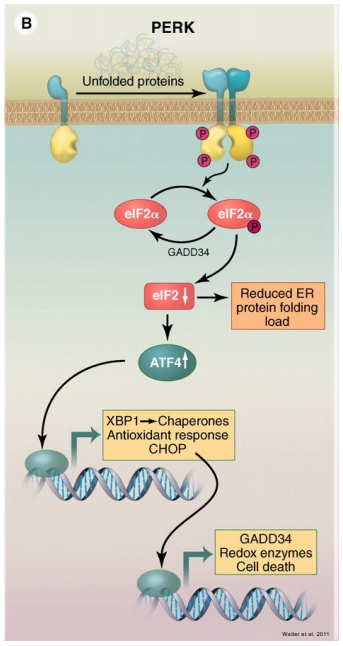
Specifically PERK kinase activity was induced in mouse embryonic fibroblast (MEFs) with PERK activator (PA). Cotreatment with the copper chelator, bathocuproine disulfonate (BCS), proved to significantly decrease PERK activity, compared to PA treated cells without BCS cotreatment, as measured by p-eIF2α levels. Furthermore, this effect was not seen upon cotreatment of Salubrinal (Sal) treated cells with BCS. As Sal induces eIF2α phosphorylation in a PERK independent manner, this demonstrates the copper chelation effect is PERK specific.

Determining the copper-dependence of PERK provides a novel mode of regulation and has numerous implications as an unconsidered variable in pathologies where PERK appears dysregulated, i.e. neurodegenerative diseases.

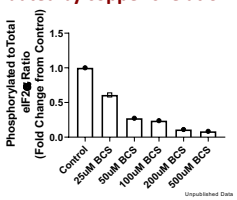
Background

PERK is the initiator of a signaling cascade that responds to ER stress and aims restore protein homeostasis





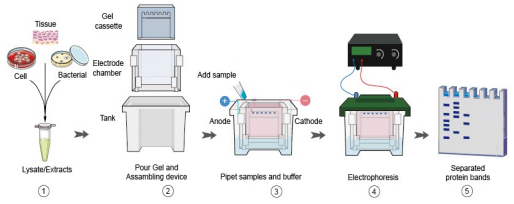
***In vitro* PERK kinase activity is attenuated by copper chelation**



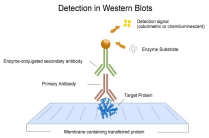
Hypothesis

Induced PERK activity is dependent on sufficient copper availability in cells.

Methods

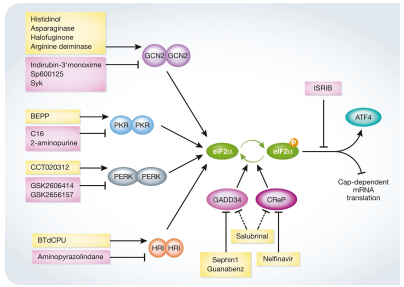


- MEFs were split at confluence and seeded at 100,000 cells per well in 6-well plates.
- Cells were treated approximately 24-47 hours after passage and lysates were harvested approximately 48 hours after passage.
- A Bradford Assay was performed on the lysates to determine protein concentrations to assure equal protein loading for Western Blot analysis.
- A Western Blot was run to quantify protein expression.



Treatments

- PERK Activator (PA) activates PERK, but its mechanisms are not well understood.
- Bathocuproine disulfonate (BCS) is a copper chelator that reduces the amount of labile copper in the cell.
- Salubrinol (Sal) prevents dephosphorylation of eif2α by inhibiting targeting of PP1 to it by GADD34 and CReP, allowing accumulation of phosphorylated eIF2α independent of PERK activity.



Results

Figure 1 (A-B): Copper chelation reduces levels of phosphorylated eIF2α induced by PERK Activator

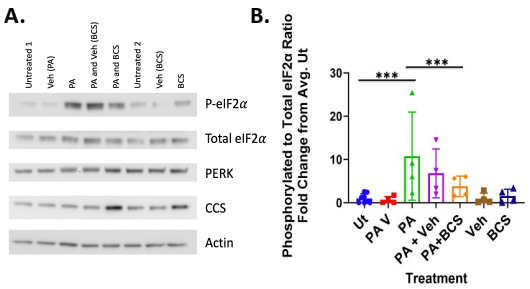


Figure 1 | MEFs treated with PA have significantly decreased eIF2α phosphorylation when cotreated with BCS, compared to controls. **A** | Western blot of whole cell lysates from MEFs treated with 500uM BCS for 24hrs and/or 10uM PA for 1hr, or equivalent dilutions of vehicle (DMSO for PA; H2O for BCS). Blot was probed with indicated antibodies, developed with Immobilon Classico Western HRP Substrate and imaged with Bio-Rad ChemiDoc imaging system. Representative images from one of four biological replicates of experiment. **B** | Quantification of the relative ratio of phosphorylated to total eIF2α for all samples in the biological replicates of the experiment shown in **A.** Statistical analysis was performed using Graphpad Prism. ANOVA gave p<0.05; Tukey's multiple comparison test was used for pairwise comparisons; ***p<0.05.

Figure 2 (A-B): Copper chelation does not reduce levels of phosphorylated eIF2α by induced Salubrinol

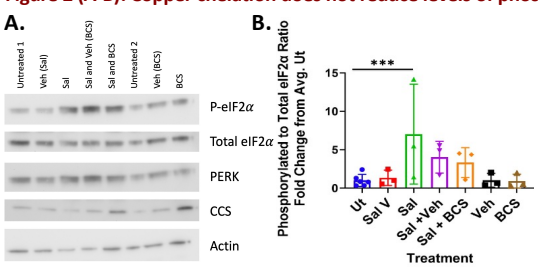


Figure 2 | MEFs treated with Sal have no significant difference in eIF2α phosphorylation when cotreated with BCS, compared to controls. **A** | Western blot of whole cell lysates from MEFs treated with 500uM BCS for 24hrs and/or 200uM Sal for 24hr, or equivalent dilutions of vehicle (DMSO). Blot was probed with indicated antibodies, developed with Immobilon Classico Western HRP Substrate and imaged with Bio-Rad ChemiDoc imaging system. Representative images from one of three biological replicates of experiment. **B** | Quantification of the relative ratio of phosphorylated to total eIF2α for all samples in the biological replicates of the experiment shown in **A.** Statistical analysis was performed using Graphpad Prism. ANOVA gave p<0.05; Tukey's multiple comparison test was used for pairwise comparisons; ***p<0.05.

Conclusions

- **Treatment of WT MEFs with PA and BCS leads to a significant reduction in the phosphorylated to total eIF2α ratio when compared to PA alone and PA & BCS vehicle treated MEFs.**
- **Treatment of WT MEFs with Sal and BCS does NOT lead to a significant reduction in the phosphorylated to total eIF2α ratio when compared to the Sal and Sal & BCS Vehicle treatments.**
- **Since PA acts directly on PERK to induce eIF2α phosphorylation, but Sal does not act via PERK, it can be concluded that the regulation of PERK activity IS copper dependent since the copper chelation had an effect only when PERK was acted upon.**

Future Directions

- Investigate this effect in a human cell model of interest, such as IPSC neurons, to see if copper chelation decreases PERK activity in these cells.
- Investigate whether Cu-ATSM, a molecule able to deliver copper directly to cells, can activate PERK.

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