How Cells Cope With Stress

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Abstract

This research will add to the current information the scientific community has on the role of stress granules. Stress granules are formed in response to the many stresses prevalent in the cell. When cells are exposed to stressors such as heat shock and oxidative stress, housekeeping translation of mRNA is halted. This process triggers the formation of stress granules which are aggregates of RNA and protein in the cytoplasm. Their purpose is to house RNA, protein and ribosomes in the cell. The cell stops translation because it is one of the most energy consuming process in the cell and when a cell is under stress, all attempts will be made to conserve energy. By conserving energy and limiting exposure of valuable cellular material to the stress, the cell has a greater chance of survival.

Purpose

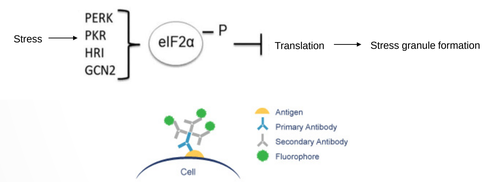
By focusing on two specific types of stress that a cell could experience and analyzing their effects on the cell, role of stress granule formation will be better understood. Cells will be treated with heat shock and oxidative stress, which are known to induce formation of stress granules in HT1080 human fibrosarcoma cells. Upon being stressed, the cells will form stress granules. One on my goals is to develop the tools required to study host response to viral infection. This experiment will involve being able to analyze the kinetics of stress granule formation in response to two specific forms of stress. Cells will be analyzed by monitoring G3BP1 activity which an integral RNA-binding protein for SG formation.. Lastly, plans are to create a Stable cell to monitor stress granule formation in real-time.

Hypothesis

Cells respond to different types of stress by inducing formation of stress granules.

Methods

- A stable cell line will be created expressing the GFP-G3BP1 plasmid
- Immunofluorescence technique will be performed on endogenous G3BP1
- There will be use of indirect immunofluorescence to target endogenous G3BP1
- For oxidative stress, cells will be treated at 0.5mM, 1 mM, 3mM, 5mM, and 10mM H_2O_2.
- Cells will then be tested at varying time points at 1 hour, 2 hours, 3 hours, 4 hours, and 5 hours, at the optimal concentration
- For the heat shock test, temperature will be kept constant at 42°C which is known to induce the heat shock pathway. Time point will be varied at 5 minutes, 10 min, 30 min, 45 min, and 60 min.

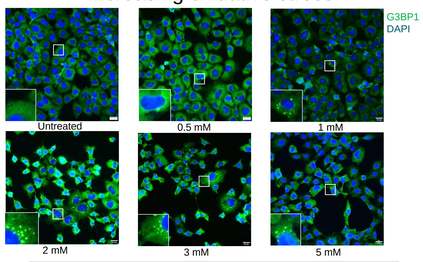


Results

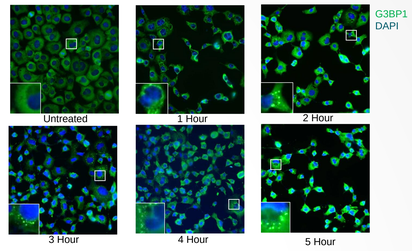
- Through monitoring endogenous G3BP1 activity, we were able to determine that 2 mM and 3 hours of H_2O_2 were optimal for inducing stress granule formation.
- Through monitoring over-expressed G3BP1 activity in the stable cell line we found that 3mM concentration and 3-hour timepoint of H_2O_2 seemed to be optimal for stress granule formation.
- For heat shock treatment, we subjected cells to 37℃. After analyzing the data monitoring endogenous and over-expressed G3BP1 we were unable to find a significant amount of cells forming stress granules at any time point of exposure and therefore further tests will be required.

Endogenous G3BP1 Activity

Stress Granule formation increases with increasing oxidative stress



Stress granule formation increases with prolonged exposure to oxidative stress



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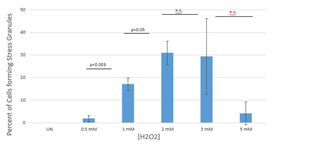
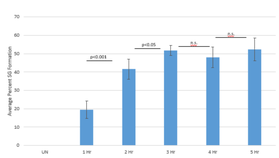


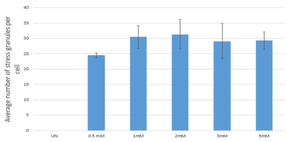
2 mM and 3 mM did not vary significantly in SG formation and future studies will determine if there is a significant difference

Stress granule formation increases with prolonged exposure to oxidative stress



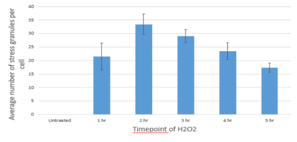
3hr, 2mM H2O2 is the optimal condition for stress granule formation

The number of stress granules forming does not increase with increasing oxidative stress



No difference in SG formation per cell between 1mM H2O2 and higher

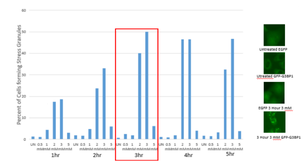
Stress granules begin to turnover with prolonged exposure to oxidative stress



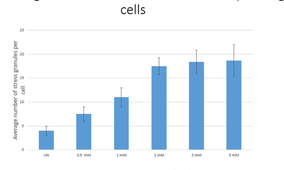
The number of stress granules increases up to 2 hours and from 2-5 hours there is a decrease

GFP-G3BP1 Overexpression Activity

Stress granule formation increases with increased oxidative stress in GFP-G3BP1-expressing cells

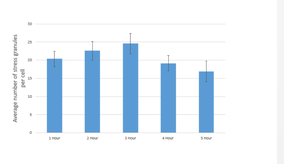


Number of stress granules forming increases with increasing oxidative stress in GFP-G3BP1 expressing cells



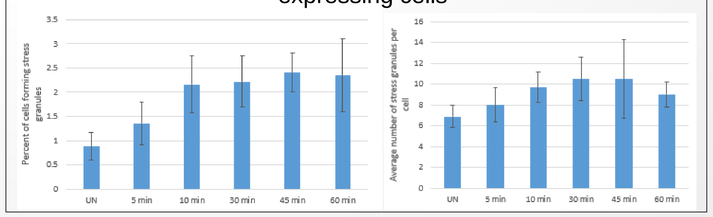
SG formation increases up to 2mM

Stress granule turnover in prolonged oxidative stress is reduced in GFP-G3BP1 expressing cells



No significant difference in the number of SG forming per cell

Heat shock induces stress granule formation in GFP-G3BP1 expressing cells



Summary

- Stress Granule formation increases with increase oxidative stress
- SG formation increases with prolonged exposure to oxidative stress
- Number of SG forming does not increase when cells undergo increased stress.
- Overexpression of G3BP1 showed similar results to endogenous activity

Future Directions

- Utilize the tools developed to study the response of viral proteins and how they promote host response to viral infection.

References

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Acknowledgments

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How Cells Cope with Stress

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The goal of this project is to understand how cells combat stress. This research will add to the current information the scientific community has on the role of stress granules. Stress granules are formed in response to the many stresses prevalent in the cell. In the body, there are many forms of stress that cells experience. I will be focusing on stress granules induced by heat shock and oxidative stress. To trigger stress granule formation, translation of messenger RNAs must come to a halt. When this happens, key stress granule protein such as G3BP1 aggregate together and form stress granules.

The first aim is to generate stable cell lines expressing GFP-G3BP1 plasmid. Whereas in the stable cell line we can monitor G3BP1 activity live in cells, to monitor endogenous G3BP1 activity we use the immunofluorescence technique. In order to track G3BP1 activity we must treat cells with hydrogen peroxide (H_2O_2) or Heat shock and determine the optimal timepoints and concentrations of the stressors that trigger stress granule formation.

For the H_2O_2 treatments, monitoring both endogenous and over-expressed GFP-G3BP1, I examined several fields for each treatment to determine the percent of cells forming stress granules per treatment. With varying concentration of H_2O_2 and by monitoring endogenous G3BP1 localization, I found that at 2 mM and 3 mM of H_2O_2, there was the highest percent of cells forming stress granules. Through monitoring endogenous and overexpressed G3BP1 activity, we were able to determine that 2 mM/3mM and 3 hours of H_2O_2 were optimal for inducing stress granule

formation. In future studies, we will be inducing stress granules using heat shock and monitor stress granule formation at different temperature.

Stress granules aggregate most of the proteins required for translation within cell to reduce energy being consumed as well as preventing unregulated translation. This project adds to current research by conducting experiments that determine how cells cope with very specific stresses such as oxidative stress and heat shock. By understanding these mechanisms, I hope to further understand the role of stress granules during viral infections.