Utilizing Live Cell Imaging in *Drosophila melanogaster* Salivary Glands to Determine if Resveratrol Treatment Activates Heat Shock Factor DNA Binding

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Abstract:

One major stress response pathway is the heat shock response (HSR), which is mediated by the transcription factor, heat shock factor (HSF). The HSR is activated in cells exposed to conditions that induce protein misfolding, such as: high heat, oxidants, and other chemical stresses. Under such stressors, HSF activates expression of the Hsp70 chaperone, which helps cells deal with protein folding stress. However, HSR activation also leads to an increase in reactive oxygen species (ROS), which can damage cellular molecules. To combat this, cells are known to utilize endogenous antioxidants to scavenge free radicals through redox reactions. Therefore, we previously examined the effect of feeding an exogenous antioxidant, resveratrol, on the ability of wildtype *Drosophila* to withstand heat stress. Treatment with 100uM and 400uM resveratrol increased the ability of the flies to withstand heat stress-induced paralysis. We hypothesize that this result occurred because the flies had increased HSF activity due to the resveratrol treatment. To examine this hypothesis, Drosophila larvae expressing HSF-GFP were dissected to obtain salivary glands. These glands contain large polytene chromosomes that allow for visualization of HSF chromosomal binding using confocal microscopy. The most easily visible binding site is an HSF doublet binding at the Hsp70 loci. Salivary glands at room temperature function as a non-heat shock (NHS) control and exhibit no binding of HSF-GFP at the Hsp70 loci. Salivary glands heated to 37C for 10, 20, 40 minutes function as the positive control and exhibit the expected Hsp70 doublet from HSF-GFP binding of the DNA. We are testing variable concentrations (100uM, 200uM, and 400uM) of resveratrol dissolved in 0.5% DMSO to determine if it activates HSF-GFP binding of the DNA in salivary glands under non-heat shock conditions. Future experiments may examine if the HSF-GFP is transcriptionally active when cells are treated with resveratrol.

Past Hypothesis:

Treatment of wildtype flies with the antioxidant resveratrol decreases their oxidative stress and improves stress induced paralysis phenotypes due to the activation of HSF.

2.

Effect of Resveratrol Treatment on a Fruit Fly Model

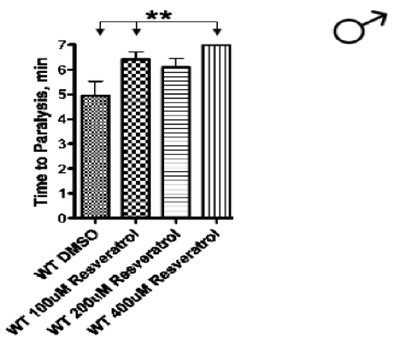

Figure 1. Resveratrol appears to improve the temperature stress sensitivity in wildtype male flies.
Wildtype (WT) males were treated with resveratrol for 5 days before being tested for temperature sensitive paralysis at 40℃. The average time to paralysis is shown above. Error bars represent standard deviation. 100uM and 400uM resveratrol treatment in WT male flies increased time to paralysis. For each condition N =10. *p<0.05 (One-way ANOVA test)

3. Conclusion:

Resveratrol treatment improves temperature stress resistance in WT male flies.

4. Current Hypothesis:

We hypothesize that this is because in WT flies, resveratrol triggers the activation of the heat shock response pathway to produce the Hsp70 chaperone protein. Hsp70 then mitigates protein misfolding, thereby improving resistance to heat induced paralysis.

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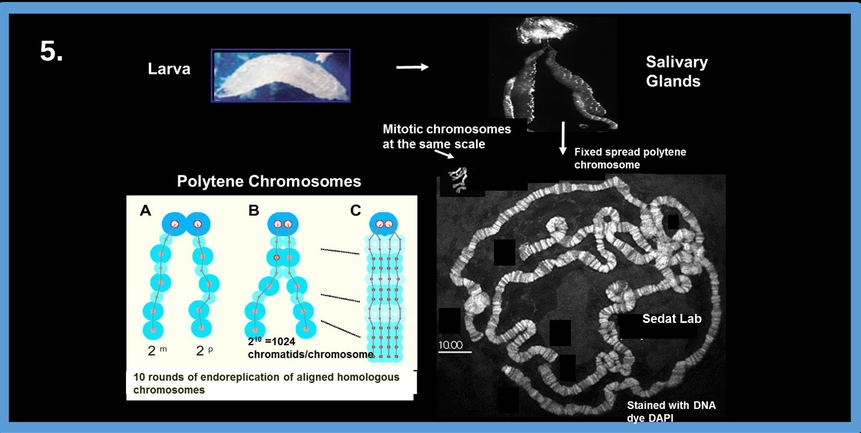


Figure 2. *Drosophila* polytene chromosomes will be used to look at HSF binding.
Drosophila polytene chromosomes provide a powerful system to visualize the recruitment of transcription factors to native genes in vivo. *Drosophila* polytene chromosomes are derived from the nuclei of larval salivary glands. Polytene chromosomes are gigantic interphase chromosomes composed of 1000 aligned chromatids. These chromatids synapse together to form amplified chromosomes that are much larger than mitotic chromosomes, as shown in the figure above. This amplification allows for the visualization of transcription factor binding at endogenous gene loci.

6. HSF Activation

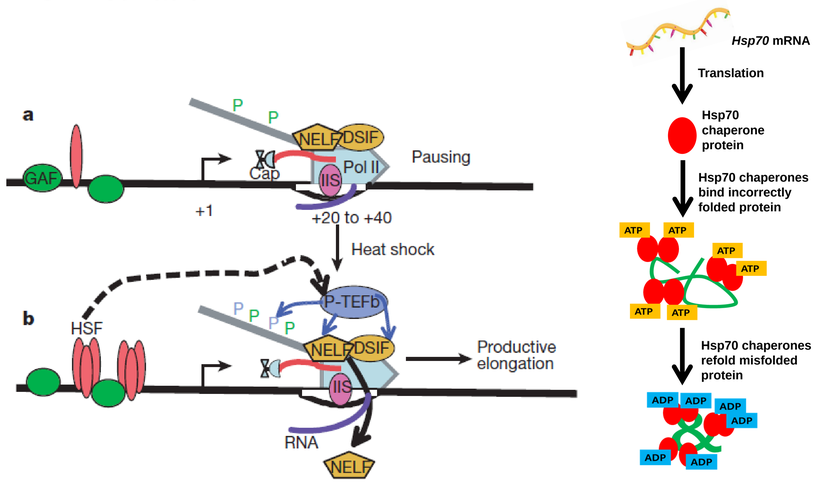


Figure 3. The heat shock response is activated through the activity of HSF, a transcription factor, that under stress conditions binds to and activates transcription of HSP70.
(A) Under non-heat shock conditions, HSF exists as a monomer that does not stably bind to the Hsp70 promoter. Upon heat shock, HSF trimerizes and stably binds to the Hsp70 promoter. This is followed by the recruitment of a battery of transcription factors that lead to the production of Hsp70 mRNA. (B) This mRNA is translated into a protein chaperone that refolds misfolded proteins.

7.

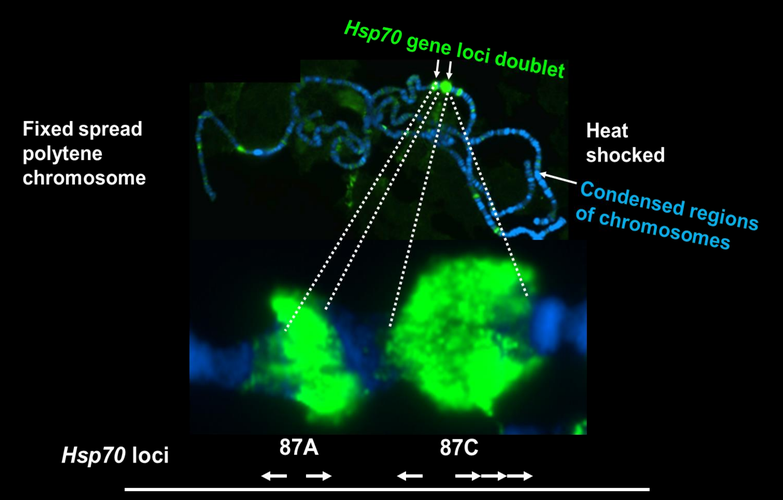


Figure 4. Localization of HSF to activated Hsp70 genes on fixed polytene chromosomes.
This image shows a fixed spread polytene chromosome that has been heat shocked and stained with an antibody against HSF shown in green and counterstained with the DNA dye DAPI in blue. Notably, you can see that HSF (in green) localizes to the decondensed or puffed (transcriptionally active) Hsp70 gene loci doublet. This doublet is known as 87A and C, 87A contains two copies of Hsp70 and 87C contains four copies of Hsp70. In contrast, the DAPI stain (in blue) localizes to condensed regions of the chromosome (transcriptionally inactive regions).

8.

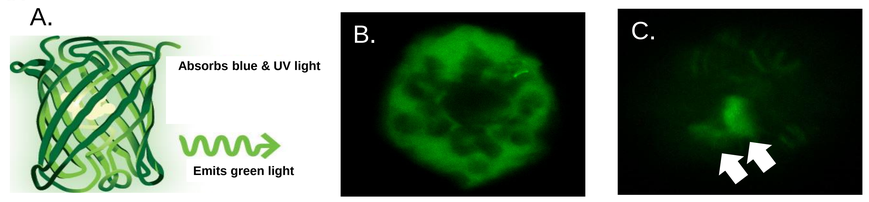
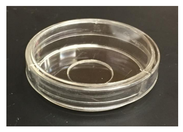
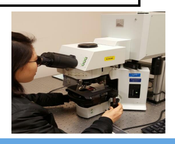


Figure 5. Localization of GFP-tagged HSF to activated Hsp70 genes on living polytene chromosomes
(A) GFP is a fluorescent protein that emits green light when excited with blue light. Drosophila HSF was tagged with GFP allowing for its visualization in living Drosophila cells using confocal microscopy (CM).(B) Under normal conditions, GFP-HSF localizes to the nucleoplasm. The polytene chromosomes appear as dark structures. (C) Under heat shock conditions, GFP-HSF is recruited to the Hsp70 loci gene doublet. The arrows highlight the Hsp70 gene loci doublet.

9. Experimental methodology for imaging HSF-GFP localization at *Hsp70* loci

1. Rear transgenic flies that expresses HSF-GFP.
2. Dissect the salivary glands from 3rd instar larvae.
3. Transfer salivary glands to the imaging dish containing desired treatment
4. Assess HSF-GFP localization at *Hsp70* loci on confocal microscope





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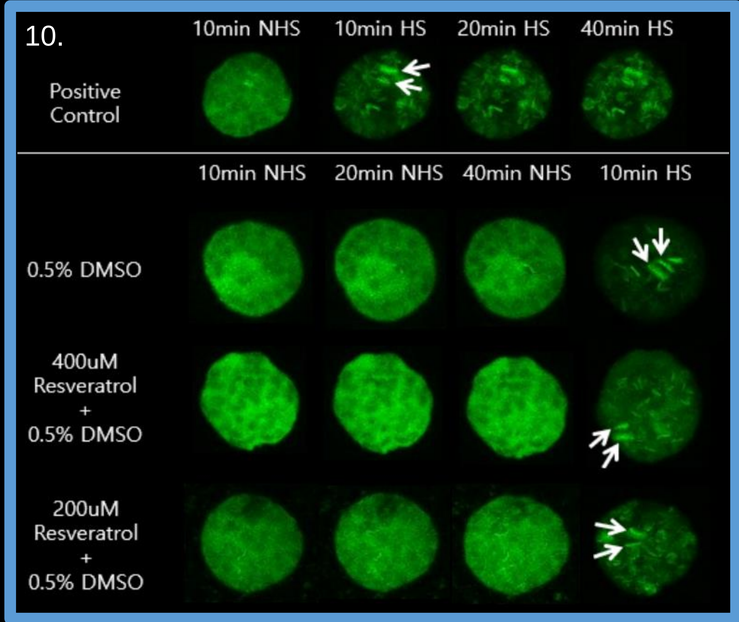

Figure 6. Resveratrol treatment does not lead to the recruitment of HSF at HSP70 loci in living polytene nuclei
The above confocal microscopy images are representative of *Drosophila melanogaster* salivary gland cells treated with or without resveratrol. For the experiment, the conditions were: the positive control (no DMSO, no resveratrol), the vehicle control (0.5% DMSO), the treatment of 400uM resveratrol dissolved in 0.5% DMSO, and the treatment of 200uM resveratrol dissolved in 0.5% DMSO. For the positive control, images were taken 10min post-treatment at room temperature (NHS) and then 10min, 20min, and 40min post-heat shock (HS). For the 0.5% DMSO, 200uM, and 400uM resveratrol treatments, images were taken 10min, 20min, and 40min post-treatment and at room temperature (NHS) and then 10min post-heat shock (HS). 14, 16, 14, and 11 salivary gland nuclei (n) were analyzed for the positive control, 0.5% DMSO, 400uM, and 200uM conditions, respectively.

11. Conclusions:

- Resveratrol treatment increases temperature stress resistance in wildtype *Drosophila melanogaster*.
- HSF-GFP does not appear to have activated DNA binding following 400uM resveratrol treatment
- HSF-GFP does not appear to have activated DNA binding following 200uM resveratrol treatment; however, testing is still in progress

Future Directions:

- Test additional dosages to confirm that HSF DNA binding is not activated at lower concentrations of resveratrol.
- Test if Hsp70 levels are increased in resveratrol-treated cells.
  - This may be due to other pathways in the cell such as the UPR (ER folding stress)
- Test if the resveratrol treatment is altering redox conditions in the cell.

12. References:

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2.) Lis, J.T. Imaging Drosophila gene activation and polymerase pausing *in vivo. Nature*. Vol 450, 199-202 (2007).
3.) Akerfelt, M. et al. Heat shock factors: integrators of cell stress, development and lifespan. *Nature Review Molecular Cell Biology.* Vol 11, 545-555 (2010).

13. Acknowledgements:

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