

HSP-70 AND THERMOPROTECTION

OVERVIEW: In this lesson, students learn about how HSP-70 protects proteins from denaturation caused by exposure to harsh conditions, and then design an experiment to test *thermoprotection* (quick acclimation to normally fatal high temperatures) in mealworms.

Ohio Science Content Benchmarks and Indicators addressed:

9-10: Life Science C. Explain the genetic mechanisms and molecular basis of inheritance.

10.5 (Heredity):

Illustrate the relationship of the structure and function of DNA to protein synthesis and the characteristics of an organism.

10.6 (Heredity):

Explain that a unit of hereditary information is called a gene, and genes may occur in different forms called alleles (e.g., gene for pea plant height has two alleles, tall and short).

Life Science D. Explain the flow of energy and the cycling of matter through biological and ecological systems (cellular, organismal, and ecological).

10.11 (Diversity and Interdependence of Life):

Explain that living organisms use matter and energy to synthesize a variety of organic molecules (e.g., proteins, carbohydrates, lipids and nucleic acids) and to drive life processes (e.g., growth, reacting to the environment, reproduction and movement).

Prior knowledge: Students should be able to explain how genes are expressed as proteins, and describe some general functions of proteins.

Equipment needed:

- Raw eggs
- Vinegar
- Mealworms
- Hot water bath or incubator
- Airtight containers to keep mealworms in (plastic screw-cap tubes are good.)

Procedures:

1. *Attention-grabber*: A few days before this lesson, soak an egg (or a few) in vinegar for two days to remove the shell. The night before, soak one of the eggs in rubbing alcohol. Have boiling water ready, or have a pre-hard-boiled egg prepared. Ask the class for predictions about what happens to both eggs. Most will know what happens to the hard-boiled egg. Few will know that rubbing alcohol has exactly the same effect (except you don't want to eat that egg.) Open the eggs and discuss the results.

2. *Discussion*:

I. Why do heat, cold, and many poisons damage living things?

- One answer is that these stresses cause their proteins to unravel, or **denature**, losing their **tertiary structure**.
- Even worse, when the denaturing shock is over, they don't re-fold into their original structure, but **misfold**, sometimes causing proteins to **agglutinate**, or clump up. In the case of the eggs that were heated and put in alcohol, the agglutination was enough to solidify the liquid yolk and white.

II. How do organisms survive stresses like this?

- One way is by turning on genes that synthesize protective molecules like **heat shock proteins**.
- The most well-understood of these is HSP-70, a heat shock protein with a molecular weight of 70 kilodaltons. HSP-70 is:
 - i. **Highly conserved**: Every single organism on earth (that's been checked for it) has the gene for HSP-70. Plants, animals, bacteria, fungi and protists. The wingless midge adults we're studying in Antarctica express HSP-70 when exposed to 86°F, which is hot for Antarctica!

- ii. **Useful in many ways:** HSP-70 can protect against heat shock, cold shock, dehydration, and heavy metal poisoning, among other things
 - iii. **A molecular chaperone:** It works by folding around other important proteins (the ones that get damaged by heat, cold, etc.) and **protecting them from being denatured.**
 - iv. **Potentially harmful if it sticks around too long:** When HSP-70 wraps around proteins, it protects them, but also keeps them from doing their jobs. If it is around at the wrong time, it can inhibit cell division, as well as keeping enzymes from doing their jobs.
- III. How does the organism know when to turn on the HSP-70 production?
- One way is simply from cues in its environment. E.g., **Thermoprotection:** if some organisms are briefly exposed to a moderately high temperature, their bodies will respond by upregulating HSP-70 (producing more of it), allowing them to survive more extreme high temps.

3. *Thermoprotection Experiment:*

- I. Instruct students to design a **thermoprotection experiment** with mealworms, the larvae of the beetle *Tenebrio molitor*.
- II. **This can be a somewhat student-directed lab: e.g., put the students in groups, tell them they have the mealworms, water baths or incubators, and containers to work with, and they need to investigate the thermoprotection of mealworms — they can try various temps for various lengths of time, approving plans with the instructor. Alternatively, the instructor can make it more structured, giving specific instructions.**
- III. Some guidelines:

- 40°C (104°F) for a couple of hours should be a mild enough temperature to initiate thermoprotection but not hot enough to kill the mealworms. 45°C (113°F) for one hour is good for a temperature that will normally hurt them. These are not exact yet (I'll work on it), so you should probably play with the temperatures and times yourself, to see what works, before you do the lab.
- Make sure the students have an experimental group and a control group.
- Make sure insects are incubated in airtight containers to prevent death by dehydration.
- 10 mealworms per sample is probably a good number, and can be easily converted into % survival.

IV. Students should keep detailed notes of procedures so that anyone could reproduce their experiment, and they should be evaluated on a lab report that includes an introduction, a hypothesis, procedures, results, and a discussion/conclusions section.