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Thermotolerance

Background

Sudden and substantial changes in the environment (shock) lead to a stress being exerted on the organism. This requires an immediate response by the organism if it is to avoid harm. Organismal reactions to shock range from behavioral responses to those at the molecular level. Many forms of environmental stress, i.e. heat, pH, salt, UV etc. cause organisms to make a series of novel proteins. The best-studied stress is heat-shock, in which an organism is exposed to a sudden increase in temperature, typically on the order of 20°C. All organisms respond to such treatment by rapidly increasing the synthesis of a specific set of proteins, collectively known as heat-shock proteins (hsp). Usually these proteins are made in unstressed cells, but at a low rate. In addition, some of the hsp appear to be synthesized following a variety of stresses, not just heat. Several of these proteins appear to be similar in all organisms and are assumed to have common functions; the most frequently seen class of proteins being those with a molecular weight in the region of 70,000 daltons. Among the proposed functions for hsp are stabilization or protection of DNA, assistance in moving proteins across membranes and 'chaperoning', moving proteins through the cell while maintaining their structure.

Initial studies with *Chlamydomonas* have shown that it also exhibits a stress response and will produce a series of heatshock proteins within a short time following exposure to the stress-inducing condition. Cells grown at 20°C show no response when the temperature is raised to 35°C but at 37°C the induction of several novel proteins is observed while uptake and incorporation of 35S remains close to normal. The effect is enhanced at 40°C and in both cases the cells appear to be synthesizing both heat shock proteins as well as those seen in cells grown at 20°C. At 42°C however uptake of 35S and the production of most proteins has virtually ceased and only a few, including the heat shock proteins are still synthesized.

The heat shock proteins include one with a molecular weight of 22 kd and a family of proteins around 70 kd. The differential behavior between 37°C and 42°C extends to cell survival. Cells held at 37°C for two hours show little or no lethality, while those held at 42°C have survival rates of <1%.

It has been known for some time that that these proteins are constitutive, and appear to play a role in the correct folding and transport of nascent proteins. As new proteins are synthesized, hsp70 molecules attach and remain with them until the protein is correctly folded and/or transported to its final destination, at which time the hsp70 is released back into the free pool. Previously, we have examined the appearance and behavior of one of these proteins, hsp70, following heat shock. We have shown that, once induced, the protein is stable for many hours and here we show that no additional induction occurs if a second heat shock is given during this time. Similarly, if cells are deflagellated, allowed four hours to regenerate new flagella, and then heat shocked, no new synthesis of hsp70 is seen. These results are consistent with the idea that anything that reduces the ratio of free:bound hsp70 results in the synthesis of additional hsp70.

Protocols

Basic heat death

Cells are grown at room temperature in liquid medium until a cell density of approximately 10^6 cell/ml. Four samples are taken. One is kept at room temperature, the others are placed at 30°C, 35°C, 40°C and 45°C for 30 minutes. All samples are serially diluted and three 0.1 ml aliquots of each dilution are spread onto S&G agar plates. After incubation for one week, the number of colonies at each concentration is determined, and the survival rate at each temperature, relative to room temperature, can be calculated. If you have time and patience other temperatures can be added: the survival cutoff is usually close to 42°C. Below this there is little or no reduction in survival; above this survival plummets to almost zero.

Preadaptation

Temperature

Cells are grown at room temperature in liquid medium until a cell density of approximately 10^6 cell/ml. Four samples are taken. One is kept at room temperature, the second is placed at 45°C for 5 minutes, the third is kept at 35°C for an hour and the last is kept at 35°C for an hour and then placed at 45°C for 5 minutes. All four samples are serially diluted and three 0.1 ml aliquots of each dilution are spread onto TAP agar plates. After incubation for one week, the number of colonies at each concentration is determined.

The usual response is that the cells exposed directly to 45°C shown almost complete lethality and never more than 1-2% survival. cells kept at 35°C for an hour show survival rates similar to room temperature and those kept at 35°C and then are exposed to 45°C show survival rates that are in between.

Deflagellation

The experiment is the same as above but cells are deflagellated rather than kept at 35°C for an hour. Again, the deflagellated cells show close to normal survival and those deflagellated and then placed at 45°C show an improvement over those placed directly at 45°C .