The Impact of Heat Stress on Queen Conch Larval Metamorphosis

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ABSTRACT

Physical factors including salinity and temperature are known to influence both invertebrate larval development and metamorphosis. A series of experiments with the queen conch, *Strombus gigas*, have shown that heat stress alone can induce larval metamorphosis. Low levels of metamorphosis are induced by exposure to temperatures 6-7 °C above culture temperatures, with maximal induction occurring at increases of 10 °C. Normal inhibitors of metamorphosis including the tyrosine kinase inhibitor genistein and the protein kinase C inhibitor phloretin do not block the heat stress response. The role of heat shock proteins in this process will be discussed.

KEY WORDS: Heat shock, larval metamorphosis, *Strombus gigas*

Impacto del Estrés por Calor en la Metamorfosis de la Larva de la Reina Concha

Se sabe que factores físicos tales como la salinidad y la temperatura, influyen tanto en el desarrollo como en la metamorfosis de larvas de invertebrados. Una serie de experimentos llevados a cabo con la reina concha, *Strombus gigas*, han demostrado que el estrés por calor, por sí solo, puede provocar metamorfosis larval. Bajos niveles de metamorfosis son inducidos mediante la exposición a temperaturas de 5-6 grados C por encima de las temperaturas de cultivo, con máxima inducción cuando los incrementos son de 10 °C. Tanto las larvas precompetentes morfológicamente, como aquéllas que parecen competentes a la metamorfosis son inducidas por el estrés por calor. Inhibidores normales de la metamorfosis, incluyendo la genisteína inhibidora de la tirosina quinasa, y la floretina inhibidora de la proteina quinasa C no bloquean la respuesta al estrés por calor. Se discutirá el papel de proteinas de shock por calor en este proceso.

PALABRAS CLAVE: Estrés por calor, metamorfosis de larvas, *Strombus gigas*

INTRODUCTION

Environmental factors such as salinity and temperature are known to influence marine invertebrate larval development and survival (see Pechenik 1987 for review, Davis 2000). Recent work indicates that these factors can also directly affect larval metamorphosis (see Pechenik et al. 1998 for review). Heat stress has been shown to induce metamorphosis in several temperate marine invertebrates (Kroiher et al. 1992, Pennington et al. 1999). In these systems, an increase in temperature alone induces metamorphosis without the addition of any other natural or artificial cue. In some cases, as with the brachiopod *Laqueus californianus*, abnormal metamorphosis can be induced by heat stress (Pennington et al. 1999). However, in other cases the induction is similar to that seen with both artificial and natural chemical inducers (Kroiher et al. 1992). In the current study, the effect of heat stress on metamorphosis of the tropical marine gastropod *Strombus gigas* was examined. Exposure temperature and timing of exposure were considered, as was the onset of competency to metamorphosis. Responses to heat stress were compared to those seen with a known inducer of conch metamorphosis, an extract of the red macroalga *Laurencia poitei* (Davis 1994a, Davis and Stoner 1994, Boettcher and Targett 1996, 1998). In order to better understand the cellular mechanisms underlying heat stress induced metamorphosis the expression of heat shock proteins (HSPs) in conch larvae and recently metamorphosed juveniles were also examined.

RESULTS AND DISCUSSION

The queen conch, *Strombus gigas*, was induced to metamorphosis at low frequencies with exposure to temperatures of 6 - 7°C above culture temperatures with maximal levels of induction seen with an increase of 10°C. Elevations in temperatures greater than 10°C led to high mortality in both larvae and recently metamorphosed juveniles. These results parallel those seen with the hydroid *Hydractinia echinata* and the tunicate *Ciona intestinalis*. For both of these species, exposure to temperatures of 10°C above culture temperatures led to normal metamorphosis with higher temperatures leading to death (Kroiher et al. 1992). The levels of queen conch metamorphosis induced by heat stress and the exposure time necessary for induction were similar to those seen in conch treated with extracts of *Laurencia poitei* (Davis 1994a, Boettcher and Targett 1998). However, heat stress appears to disrupt the normal signaling pathway involved in conch metamorphosis as shown by the results of normal inhibitors of conch metamorphosis (Boettcher, unpublished data).

Heat stress induced metamorphosis in queen conch larvae was coupled to changes in the expression of heat shock proteins. Conch larvae exposed to heat stress consistently showed an increase in HSP 90 expression over time. A distinct increase in expression was seen at four hours of exposure with another increase at 24 hours post exposure. This pattern appears to be comparable to that seen for both HSP 70 and HSP 60, although the expression of these proteins has not been as well

studied. Kroiher et al. (1992) also saw increases in expression of proteins corresponding to known heat shock proteins in larval *H. echinata* exposed to heat stress. Further studies examining expression levels of HSPs in heat stress induced larvae, and in those induced to metamorphose by natural and chemical inducers of metamorphosis, will contribute to our understanding of mechanisms controlling normal metamorphosis and the impact of environmental stresses on this process (Feder 1999, Hofmann 1999, Lewis et al. 1999).

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