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Arginine kinase in the cladoceran *Daphnia magna*: cDNA sequencing and expression is associated with resistance to toxic *Microcystis*

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ABSTRACT

Nutrient loading derived from anthropogenic activities into lakes have increased the frequency, severity and duration of toxic cyanobacterial blooms around the world. Although herbivorous zooplankton are generally considered to be unable to control toxic cyanobacteria, populations of some zooplankton, including *Daphnia*, have been shown to locally adapt to toxic cyanobacteria and suppress cyanobacterial bloom formation. However, little is known about the physiology of zooplankton behind this phenomenon. One possible explanation is that some zooplankton may induce more tolerance by elevating energy production, thereby adding more energy allocation to detoxification expenditure. It is assumed that arginine kinase (AK) serves as a core in temporal and spatial adenosine triphosphate (ATP) buffering in cells with high fluctuating energy requirements. To test this hypothesis, we studied the energetic response of a single *Daphnia magna* clone exposed to a toxic strain of *Microcystis aeruginosa*, PCC7806. Arginine kinase of *D. magna* (Dm-AK) was successfully cloned. An ATP-gua PtransN domain which was described as a guanidine substrate specificity domain and an ATP-gua Ptrans domain which was responsible for binding ATP were both identified in the Dm-AK. Phylogenetic analysis of AKs in a range of arthropod taxa suggested that Dm-AK was as dissimilar to other crustaceans as it was to insects. Dm-AK transcript level and ATP content in the presence of *M. aeruginosa* were significantly lower than those in the control diet containing only the nutritious chlorophyte, *Scenedesmus obliquus*, whereas the two parameters in the neonates whose mothers had been previously exposed to *M. aeruginosa* were significantly higher than those of mothers fed with pure *S. obliquus*. These findings suggest that Dm-AK might play an essential role in the coupling of energy production and utilization and the tolerance of *D. magna* to toxic cyanobacteria.

1. Introduction

Concomitant with the eutrophication of lakes, nuisance cyanobacterial blooms have become increasingly frequent and widespread for several decades (Chislock et al., 2013a; Merel et al., 2013). In addition to worsening the quality of water resources for human use, high cyanobacterial abundances may also have considerable negative impacts on herbivorous zooplankton, including the important generalist zooplankton genus, *Daphnia* (Reynolds, 1994; Wilson et al., 2006; Yang et al., 2011, 2012).

Cyanobacteria have several characteristics that make them insufficient as a food source for zooplankton. For example, grazing-resistant forms, such as large filaments and colonies, may mechanically interfere with feeding (De Bernardi et al., 1990; Yang et al., 2006), while the low content of essential fatty acids in cyanobacteria can suppress growth rates of zooplankton (Von Elert and Wolffrom, 2001). Moreover, some cyanobacterial genera can produce toxins (e.g. microcystins; MCs) (Catherine et al., 2013) that can inhibit zooplankton by reducing filtering rates or causing rapid death (DeMott et al., 1991; Lürling and Beekman, 2006; Xiang et al., 2010). Despite disagreement on the causal factors, cyanobacteria in the diet typically reduced zooplankton growth and reproduction (Lürling, 2003; Martin-Creuzburg et al., 2005; Wilson et al., 2006; Lyu et al., 2013a,b,c; Hochmuth and De Schamphelaere, 2014), which can lead to zooplankton population declines that can reduce energy transfer efficiency in aquatic food-webs (Miner et al., 2012; Ger et al., 2014).

To withstand toxic cyanobacteria, some *Daphnia* species have been shown to adapt to toxic cyanobacteria (Gustafsson et al., 2005; Guo and Xie, 2006; Wilson and Hay, 2007; Chislock et al., 2013b; Jiang et al., 2013). For example, Hairston et al. (1999) first showed the evolution of resistance in *Daphnia galeata* to

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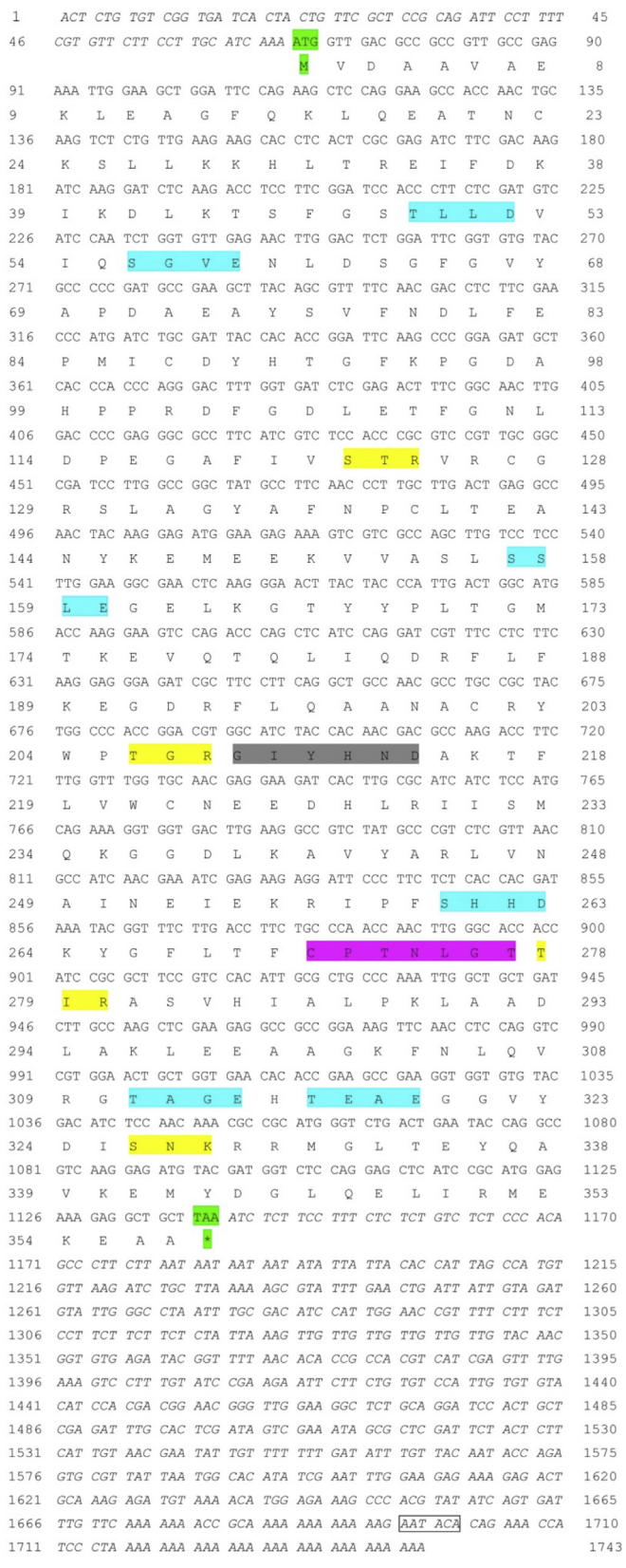

Fig. 1. Nucleotide and deduced amino acid sequences of *D. magna* arginine kinase (Dm-AK). The nucleotide sequence is numbered from 5′ end, and the single letter amino acid code is shown below the corresponding codon. The start codon (ATG) and the end codon (TAA) highlighted in fluorescent green color. Potential ATP: guanido phosphotransferases active site highlighted in pink color. Four protein kinase C phosphorylation sites are highlighted in yellow color. One N-myristoylation site is highlighted in gray color. Six casein kinase II phosphorylation sites are highlighted in turquoise color. The termination code is marked with an asterisk. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

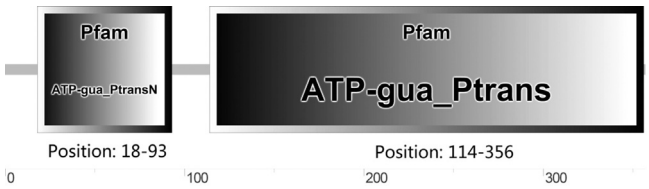

Fig. 2. The architecture of deduced Dm-AK amino acid (356 amino acids). An ATP-gua PtransN and an ATP-gua Ptrans domain were at the position 18–93 and 114–356, respectively. Protein domains were predicted by SMART program.

cyanobacteria by contrasting the performance of *Daphnia* clones hatched from contrasting periods in the eutrophication history of Lake Constance to diets lacking or containing toxic *Microcystis aeruginosa*. Since then, related studies have shown that adaptations in *Daphnia* to toxic cyanobacteria occur across lakes that vary in productivity and abundance of cyanobacteria (Sarnelle and Wilson, 2005) and that the effects of *Daphnia* resistance on algal abundance can be as important as the presence or absence of *Daphnia* (Sarnelle and Wilson, 2005; Chislock et al., 2013b). Even short-term exposure to *M. aeruginosa* can quickly induce resistance to cyanobacteria in *Daphnia* (Gustafsson and Hansson, 2004) or cause feeding selectivity to reduce the ingestion of cyanobacteria (Tillmanns et al., 2011). One possible explanation for these observations is that tolerant zooplankton may elevate energy production, thereby adding more energy allocation to detoxification expenditure (Bossuyt and Janssen, 2004; Ortiz-Rodríguez and Wiegand, 2010; Bergman Filho et al., 2011). Recent results on the gene expression of key enzymes of carbohydrate and protein metabolism showed disruptions on the energy producing pathways of *D. magna* exposed to *M. aeruginosa*. These findings suggest that important functions of energy status to maintain metabolic homeostasis may compete with energy deficiency and toxicity due to feeding on cyanobacteria (Schwarzenberger et al., 2009; Asselman et al., 2012).

In invertebrates, arginine kinase (ATP: arginine N-phosphotransferase, EC 2.7.3.3; AK) which is analogous to the creatine kinase (CK) reaction in vertebrates, serves as a core in temporal and spatial ATP buffering in cells with high, fluctuating energy requirements (muscle, nerves, etc.) (Uda et al., 2006). AK catalyzes the reversible substrate-level phosphorylation of arginine by MgATP to form phosphoarginine and MgADP, thereby regenerating ATP during bursts of cellular activity (Zhou et al., 1998; Alonso et al., 2001). A growing literature has revealed an evolutionary relationship between invertebrate AK and vertebrate CK and their analogous metabolic roles (Bogdan, 2001). AK has been found to synthesize phosphagen in one direction when ATP supply is abundant, and in the opposite direction to mediate the rapid breakdown of phosphagen during acute stress response. In recent studies, it was elucidated that the expression of AK correlated closely with salinity change in blue crab, *Callinectes sapidus* (Kinsey and Lee, 2003), acclimation to cadmium in crab, *Eriocheir sinensis* (Silvestre et al., 2006), the exposure to lead in yabby, *Cherax destructor* (Morris et al., 2005), and hypoxic stress in the kuruma prawn *Marsupenaeus japonicas* (Abe et al., 2007). Therefore, AK is believed to play a crucial role in environmental stress responses in terms of regulating energy production and utilization in crustaceans.

However, little is known about AK characterization and functions of *Daphnia* which have been shown to locally adapt to