# *Cg*miR307 involved in the regulation of Nrf2-dependent oxidative response in the Pacific oyster *Crassostrea gigas* under high-temperature stress

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**ABSTRACT：** miRNA, a type of endogenous small non-coding RNA, is involved in the response to various environmental stresses, through post-transcriptional regulation. In the present study, the role of *Cg*miR307 in the regulation of oxidative response under high-temperature stress by targeting *Cg*Nrf2 was investigated in the Pacific oyster Crassostrea gigas. The binding sites of *Cg*miR307 were predicted at 1,799-1,818 bp in the 3'-UTR of *Cg*Nrf2, and the binding activity of *Cg*miR307 with *Cg*Nrf2 was further proved by the dual-luciferase reporter assay. The expression levels of *Cg*miR307 and *Cg*Nrf2 in gill were significantly higher than in other tissues, and exhibited significant fluctuations and variations after high-temperature stress at 28℃. After *Cg*miR307 agomir injection and high-temperature stress, the expression levels of *Cg*Nrf2, *Cg*SOD and *Cg*CAT in gill, the activities of SOD and CAT and T-AOC decreased significantly, which were 0.63-fold ($p < 0.01$), 0.32-fold ($p < 0.05$), 0.35-fold ($p < 0.001$), 0.69-fold ($p < 0.01$), 0.17-fold ($p < 0.001$) and 0.43-fold ($p < 0.001$) of that in the control group, respectively, while MDA content significantly increased to 1.57-fold ($p < 0.001$) of that in the control group. After *Cg*miR307 antagomir injection and high-temperature stress, the expression levels of *Cg*Nrf2, *Cg*SOD and *Cg*CAT in gill, the activities of SOD and CAT and T-AOC increased significantly compared to that in the control groups, respectively, while MDA content significantly decreased. These results demonstrated that *Cg*miR307 was involved in the regulation of oxidative response by inhibiting the expression of *Cg*Nrf2 under high-temperature stress. These findings contributed to the understanding of miRNA regulation of Nrf2 in the response to high-temperature stress in molluscs.

## Results

### 1. *Cg*miR307 sequence and target prediction

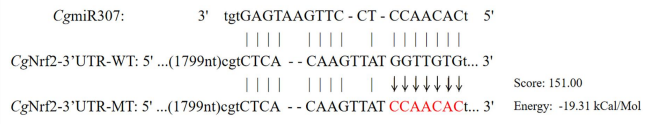


Fig. 1. *Cg*miR307 targeted *Cg*Nrf2. Target genes *Cg*Nrf2 3'-UTR on *Cg*miR307 conservative seed binding site. The mutant sequences used in the subsequent dual luciferase reporter are shown in red.

### 2. Phylogenetic characterization of *Cg*Nrf2

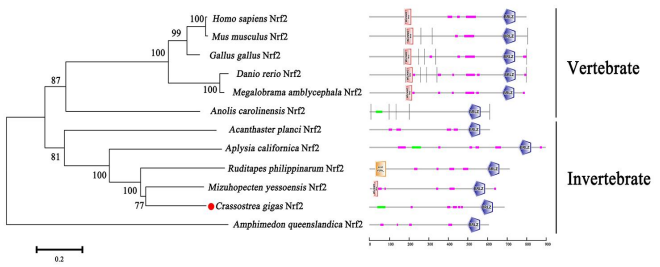


Fig. 2. Phylogenetic analysis of the Nrf2s in vertebrates and invertebrates.

### 3. The expression levels of *Cg*miR307 in different tissues and after high-temperature stress

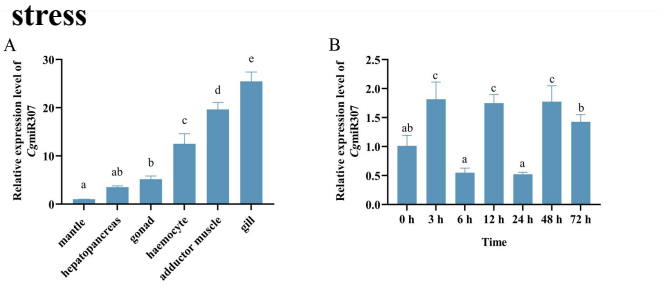


Fig. 3. The expression of *Cg*miR307 in different tissues (A) and in gills after high-temperature stress (B).

### 4. The expression levels of *Cg*Nrf2 in different tissues and in gill after high-temperature stress

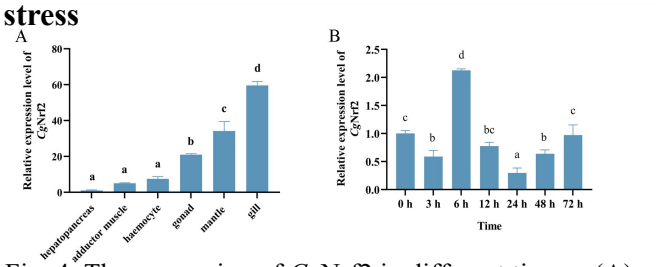


Fig. 4. The expression of *Cg*Nrf2 in different tissues (A) and in gills after high-temperature stress (B).

### 5. The interaction between *Cg*miR307 and *Cg*Nrf2 3'-UTR

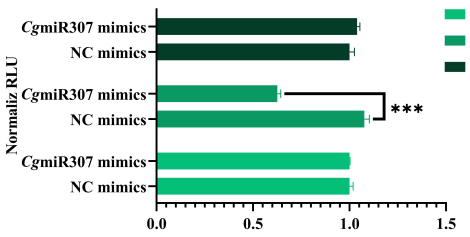


Fig. 5. The targeting of *Cg*miR307 on the 3'-UTR region of *Cg*Nrf2 in *vitro*. *Cg*Nrf2 3'-UTR luciferase reporter assay was conducted in HEK293T cells to investigate its potential interaction with *Cg*miR307. (***: $p < 0.001$).

### 6. Changes of the expression levels of *Cg*miR307 and *Cg*Nrf2, and the parameters of oxidative response after the injection of *Cg*miR307 agomir in *vivo*

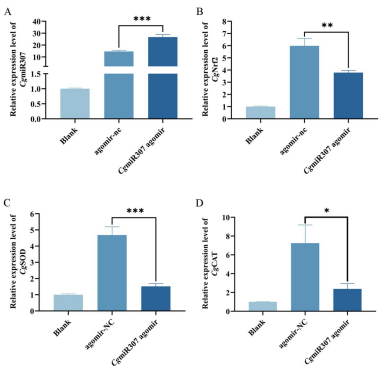


Fig. 6. The mRNA expressions of *Cg*miR307 (A), *Cg*Nrf2 (B), and the antioxidant genes *Cg*SOD (C) and *Cg*CAT (D) after gain-of-function assay of *Cg*miR307 in *vivo*. (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

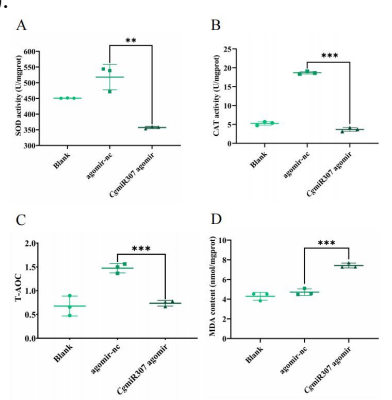


Fig. 7. The parameters of oxidative stress after gain-of-function assay of *Cg*miR307. A: SOD activity, B: CAT activity, C: T-AOC, D: MDA content (**: $p < 0.01$; ***: $p < 0.001$).

### 7. Changes of the expression levels of *Cg*miR307 and *Cg*Nrf2, and the parameters of oxidative response after the injection of *Cg*miR307 antagomir in *vivo*

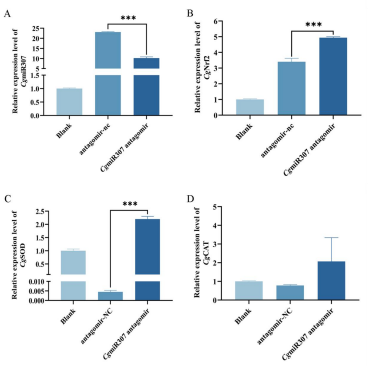


Fig. 8. The mRNA expressions of *Cg*miR307 (A), *Cg*Nrf2 (B), and the antioxidant genes *Cg*SOD (C) and *Cg*CAT (D) after lost-of-function assay of *Cg*miR307 in *vivo*. (***: $p < 0.001$).

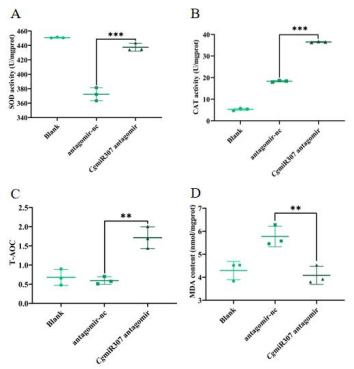


Fig. 9. The parameters of oxidative stress after lost-of-function assay of *Cg*miR307. A: SOD activity, B: CAT activity, C: T-AOC, D: MDA content (**: $p < 0.01$; ***: $p < 0.001$).

## Conclusion

The results of this study clearly demonstrated that *Cg*miR307 acted as an upstream regulatory miRNA of *Cg*Nrf2, and was able to modulate the antioxidant capacity and oxidative response levels of oysters by inhibiting the mRNA expression level of *Cg*Nrf2 under high-temperature stress.