Micrornas modulate circadian clock genes to enhance zebrafish cold tolerance

Professor Shyh-Jye Lee of the Department of Life Science, who also serves as the director of the Research Center for Developmental Biology and Regenerative Medicine, led his team in demonstrating that microRNAs (miRNAs) regulate the cold responses of fish by fine-tuning circadian clock genes. Using genomics approaches, the team discovered that miRNA-dependent circadian clock genes help zebrafish larvae to withstand cold stress. This research provides insights that may help to reduce massive fishery losses during cold fronts in Taiwan. This study was published in the journal *BioMed Central Genomics* in November 2016 [1].

In winter, cold fronts often lead to considerable numbers of deaths of aquatic animals andcause huge losses for Taiwan's aquaculture sector. However, there are currently no effective ways of managing this cold damage. Cold damage occurs under rapid decreases in environmental temperatures, causing a fast drop in the core body temperature of fish and a series of physiological and stress responses known as cold shock.

Although cold shock is known to cause death and other physiological responses in fish, [2,3] the molecular regulatory mechanisms underlying these changes remain unknown. Professor Lee reasoned that miRNAs, which are small non-coding RNAs, may also be affected and contribute to the regulation of cold shock because miRNAs are involved in development, cell division, and metabolism. miRNAs are also important in regulating environmental stresses, such as temperature changes. [4]

miRNAs function by blocking the expression of their target mRNAs. Using zebrafish larvae as a model, Professor Lee and his collaborator, Professor Sunny Sun at the National Cheng Kung University Medical College, led their teams in performing small RNA-seq and RNA-seq analyses to gain a global understanding of the changes in miRNA and mRNA expression in response to cold shock. Small RNA-seq and RNA-seq are high-throughput, next-generation sequencing methods used for miRNAome and transcriptome profiling.

The miRNAome profiling analyses revealed 29 up-regulated and 26 down-regulated miRNAs upon cold shock, and the transcriptome profiling analysesconfirmed that many cold-affected miRNA target genes are also influenced. Further analyses showed that these miRNAs and mRNAs are involved in many cellular physiological responses, such as the regulation of circadian rhythms, as evidenced by the tenfold overexpression of the *per2* gene, which is one of the core clock genes. Moreover, zebrafish larvae overexpressing *per2* showed significantly improved recovery of motility after cold shock compared with the control group (Fig. 1). In addition, glucose concentrations in the *per2*-overexpressing larvae were higher than those in the control group, indicating that this gene may modulate glucose metabolism under cold shock to increase cold tolerance.

In conclusion, miRNAs regulate genomic plasticity during cold shock. The core clock gene *per2* helps zebrafish larvae to withstand cold temperatures, presumably via the fine-tuning of the associated miRNA *dre-mir-29b*. Therefore, the appropriate modulation of circadian clock genes may represent an effective strategy for managing cold challenges in aquaculture.

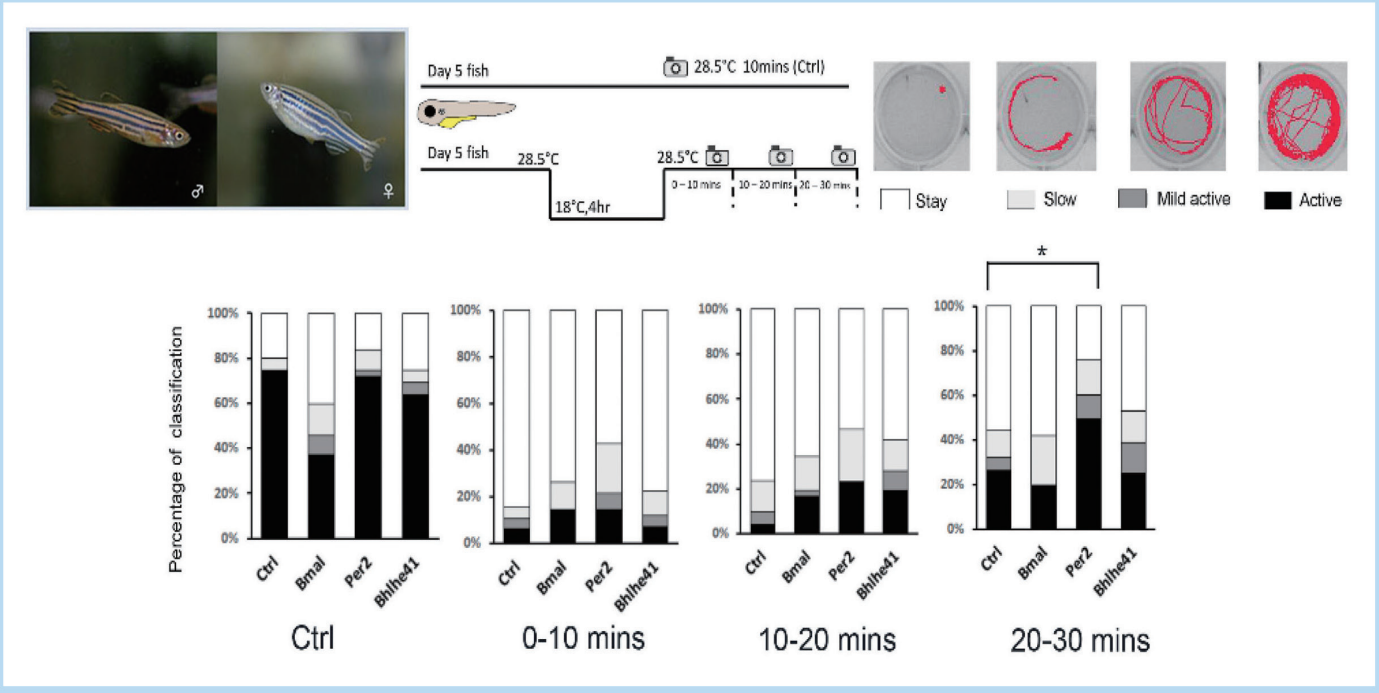

Figure 1. An adult zebrafish pair, the cold shock scheme and the recovery of motility (Modified from Reference 1).

References

1. I-Chen Hung, Yu-Chuan Hsiao, H. Sunny Sun, Tsung-Ming Chen* and Shyh-Jye Lee*, (2016). MicroRNAs regulate gene plasticity during cold shock in zebrafish larvae. *BMC genomics*, 17, 922. Published online 15 November 2016. DOI:10.1186/s12864-016-3239-4.

2. John R. Brett, (1971). Energetic Responses of Salmon to Temperature. A Study of Some Thermal Relations in the Physiology and Freshwater Ecology of Sockeye Salmon (Oncorhynchus nerka). *American Zoologist* 11, 99-113. DOI:10.2307/3881652.

3. M. R. Donaldson, S. J. Cooke, D. A. Patterson and J. S. Macdonald, (2008). Cold shock and fish. *Journal of Fish Biology*, 73, 1491-1530. DOI:10.1111/j.1095-8649.2008.02061.x.

4. Dong Ci, Yuepeng Song, Min Tian and Deqiang Zhang*, (2015). Methylation of miRNA genes in the response to temperature stress in *Populus simonii*. *Frontiers in Plant Science*, 6, 921.Published online 30 October 2015. DOI:10.3389/fpls.2015.00921.

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