V. Discussions

The swimming ability of a fish is strongly correlated with muscle contraction during thermal acclimation (Swank DM & Rome LC, 2001). This process depends heavily on energy metabolism, which is tightly regulated by CK isoenzymes. High-level expression of M3-CK was detected in transgenic fish indicating that the enhanced swimming ability of transgenic zebrafish under the adverse condition could be likely sustained by this transgene. The creatine pool, ATP concentration and the activity of CK regulate the homeostasis of the muscle cell, which in turn maintains the energy reservation for muscle contraction (Boutilier *et al.*, 1997).

Thus, the active swimming behavior of the transgenic fish at low temperature is understandably. The muscle contractions at low temperature rely on the maximum capacity of the chemical energy supplemented by the creatine pool and the balance of ATP and phosphocreatine, which is reversibly transferred by carp M3-CK. We have monitored the functional property of carp M3-CK in transgenic zebrafish and assessed its performance in the muscle energy over long periods at low temperatures, and have concluded that the observation supported our hypothesis that the transgene has increased the cold tolerance in the zebrafish.

Indeed, the significant improvement in cold tolerance in the transgenic zebrafish at otherwise intolerable low temperatures suggests that the carp M3-CK gene have been functionally integrated into the energy metabolism of the transgenic fish. Physiologically, the carp M3-CK enzyme could exhibit its low temperature preference by altering the endogenous energy metabolic pathways to sustain muscle contraction

as depicted in the phenotypic expression of the transgenic zebrafish.

On the basis of the swimming behavior between the transgenic and wild type zebrafish, we propose that the movement is regulated by a two-step biochemical reaction. First, the consumption of ATP for muscle contraction is primarily determined by the efficiency of ATP synthesis in fish. Second, the homeostasis of the muscle creatine and phosphocreatine pool is determined by the dynamic equilibrium mediated by the CK isoenzyme in the creatine biosynthesis pathway (Udvadia AJ & Linney E, 2003). It appears that, the homeostasis is well maintained in the M3-CK transgenic zebrafish, so that there is a mechanism for rapid energy production to meet different environmental requirements (Table 7) without the need to altering other metabolic pathways (Sun *et al.*, 1998).

The M3CK promoter also maintained the swimming ability in the M1-CK transgenic zebrafish, and improved the mortality in low water temperature. There is something interesting for us. Maybe on the M3CK promoter, it has some cold inducible or feedback control transcription factor binding site, and it is regulated by ATP/ADP ratio or some protein maintained cellular homeostasis. Thus, M3-CK promoter can promote M1-CK synthesis; the quantity of M1-CK is more over than normal condition in the M1-CK transgenic zebrafish. It consumed more energy to synthesis dysfunction protein; M1M1-CK maintains lower activity than M3M3-CK at lower temperature. Surplus relative activity of M1M1-CK is less 20% in low temperature.

While the constitutively active M3-CK in transgenic zebrafish to eventually exhaust all the energy stored in the phosphocreatine without replenishing the phosphocreatine pool product from other energy generation pathways (Gorselink *et al.*, 2001), our present findings suggest that the constitutively expressed carp M3-CK is well adapted in the

transgenic zebrafish. Moreover, the M3-CK enzyme could effectively compensate a rapid energy demand at low temperature in muscle by transferring phosphocreatine to ATP (Roussel *et al.*, 1998). Thus, carp M3-CK supports the active swimming behavior of transgenic fish at the unfavorable temperature. This is the first demonstration of enhancing zebrafish's motility and survivability under adverse conditions by introducing a heterologous carp M3-CK gene coupling to energy metabolism in fish muscle, an important step towards enhancing cold tolerance in subtropical fish.