

Our results suggested that the environment of south China is suitable for the growth and reproduce of *R. chensinensis*. The main results were as follows:

1. The ratio of mating of adult *R. chensinensis* were 84% in south China, and the ratio of spawning were 68%, the survival rate were 42%, and the hatch rate of oosperm were 56%.
2. The temperature in south China is higher than that in east-north China, which reduces the time of the embryonic development of *R. chensinensis*. In natural condition, the early embryonic development of *R. chensinensis* required 131–155 h and the postembryonic development required 28–30 days. Totally, the embryonic development required 32–35 days, which was less about one month than that in east-north China.
3. The higher temperature expedited the embryonic development, but it did not affect the normal development of embryo. During the period of embryonic development, there almost no unexpected died and misshapen individual.
4. The results of our study on the dissection of tadpole and larval frog and the organic slice of gonad confirm that the early development of gonad was in good condition. The processes of the emergence of original gonad and the split of germ cell etc were on the rails.
5. With the reduce of the time of embryonic development of *R. chensinensis*, its original gonad came into being ahead of time, but the differentiation of gonad was behind the schedule.
6. The pattern of gonadal sex differentiation of *R. chensinensis* is the differentiated type.
7. The results we had reported above not only enrich the development biology but also provide the foundation of theory and practice for introducing the *R. chensinensis* to south China.

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MOLECULAR CLONING AND EVOLUTIONARY ANALYSIS OF HYPOXIA-INDUCIBLE FACTOR 1 ALPHA, AND EPO RESPONSIVENESS UNDER HYPOXIA IN THE TIBETAN VERTEBRATES *PANTHOLOPS HODGSONI*, *MYOSPALAX BAILEYI*, *MYOSPALAX CANSUS*, *MICROTUS OECONOMUS* AND *GYMNOCYPRIS PRZEWALSKII*

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Hypoxia-inducible factor 1 α (HIF-1 α) is an essential mediator of oxygen homeostasis. The Tibetan antelope (*P. hodgsoni*), plateau pika (*O. curzoniae*), plateau zokor (*M. baileyi*, *M. cansus*), root vole (*M. oecomus*), and naked carp (*G. przewalskii*) are native vertebrates of the Qinghai–Tibetan plateau and well acclimatized to hypoxia. To better understand their adaptive mechanisms to hypoxia, cDNA encoding HIF-1 α was isolated and characterized. The deduced HIF-1 α sequences of *P. hodgsoni*, *M. oecomus*, *M. baileyi* and *M. cansus* showed 90–99% identity with those of the human, rat, and yak; *G. przewalskii* had 57–89% identity with rainbow trout and common carp. The conservational and phylogenetic clustering of the HIF-1 α sequences was consistent with vertebrate classification. We estimated codons under positive selection. All positive selection sites were outside the key domain, but between the key domains of TAD-N and TAD-C (607L, 611T, 607E, 622D, 623E, 624L, 627V, 632M, 645T, and 663Q), and another was 8E in *M. baileyi*. The evidence shows that plateau animals have specializations for hypoxia linked to changes of Tibetan Plateau ecological environment. HIF-1 increased in the cortex and liver of the mice, *M. baileyi*, and *M. oecomus* under hypoxia, but not in *O. curzoniae*; EPO increased in the cortex and kidney of mice under hypoxia, but only in the kidney of *M. oecomus*. In addition, under CoCl₂-hypoxia, EPO increased in the cortex and kidney of three Tibetan mammals, but not in mice. The differences in HIF and EPO between Tibetan animals and lowland mice suggest that diverse strategies are involved in hypoxia. HIF-1 may play a role in mice and *M. baileyi* but not in *O. curzoniae*. This work was supported by the

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NF- κ B FUNCTIONS THE DIVERSITY OF CELLULAR IGF-I/IGFBP-1 EXPRESSION BY HYPOXIA IN TIBETAN PLATEAU MAMMALS

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Ochotona curzoniae, *Microtus oecomus* and *Myospalax baileyi* are all native mammals that reside at Qinghai–Tibetan plateau in China and well acclimatized to environmental hypoxia. The present paper addresses the NF- κ B's, a nuclear transcriptional factor, involvement in hypoxia stress-induced diversity of IGF-I/IGFBP-1 expression in hepatic and brain cells of Tibetan Plateau mammals. The IGF-I/IGFBP-1 from the prefrontal cortex and the liver cells was tested 6 h after hypoxia exposure (by CoCl₂ injection *i.p.* 20, 40 mg/kg or by normobaric hypoxia, 16.0%, 10.8%, 8.0% O₂) of the Plateau native mammals and mice. PDTC, an inhibitor of NF- κ B, was used and preinjected before the hypoxia to evaluated NF- κ B action. The results showed that 1) the IGF-I expression in mice hepatic cells of *M. oecomus* and *M. baileyi* markedly increased after the hypoxia exposure, but there was no response in the liver of *O. curzoniae*; 2) the IGFBP-1 expression in mice hepatic cells of *O. curzoniae* and *M. baileyi* markedly enhanced, but no response occurred in *M. oecomus* after the hypoxia; 3) PDTC pretreated before hypoxia reversed the hypoxia-enhanced IGF-I in *M. oecomus* and *M. baileyi*; 4) PDTC treatment also reversed the hypoxia-enhanced IGFBP-1 in *O. curzoniae* and *M. baileyi*; 5) hypoxia increased the IGF-I mRNA in brain of *M. oecomus* and *O. curzoniae* but not of mice; 6) hypoxia did not induce changes of IGF-I levels in the brain cells of both plateau mammals and laboratory mice. The data suggest that 1) different pattern in IGF-I/IGFBP-1 expression induced by hypoxia represents a diversities in hormone regulation and cell protection from damage in Tibetan native mammals; 2) NF- κ B mediates the transcription of IGF-I/IGFBP-1 in liver cells subjecting to hypoxia; Together, the diversity of target-gene phenotype expression may contribute to the multi-model in cell protection from hypoxia damage.

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A TRANSCRIPTOME SCHEME OF GILL REVEALS THE COLD ACCLIMATION STRATEGIES IN ZEBRAFISH (*DANIO RERIO*)

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Global analysis of gene expression using functional genomics approaches is a revolutionizing technique for molecular physiological studies. DNA microarray is a powerful technique to identify the differential expressed genes among two or more populations as an indirect measure of functional changes of the related proteins. Fish are ectodermic vertebrates and have some advantages to study the relationships between environmental temperature and their genome. The purpose of this study was to investigate the effects of low temperature on the functions and differentiation of gill cells. We used microarray technology to compare the gene expression profiles in gill cells between acute and chronic treatments of low temperature. Adult zebrafish were acclimated to a low temperature of 12 °C for 1 (1-d) and 30 d (30-d), and the gene expression patterns of gill were compared to control groups (28 °C). The transcriptome profiles of 1-d and 30-d treatments were extreme different, only 2 genes were conserved, suggesting that zebrafish may utilize distinct strategies for short- and long-term cold acclimation. Several ionoregulation-related genes were identified from these profiles and we further investigated the role of ionoregulatory genes in zebrafish gills during cold acclimation. Broad functions of ionocytes related genes were induced by cold, indicating that terminal differentiation and function of ionocytes were stimulated to recover the cold-induced imbalance of ion and acid/

base. We also found that both cell proliferation and apoptosis were suppressed by cold treatment implying an extension of cell life span after cold acclimation. These results suggest that gill ionocytes may extend their lifespan by delaying natural cell death, and gills may sustain their functions by yielding mature ionocytes from preexisting undifferentiated progenitors. Hence, our studies on gill transcriptome provide new insights into the cellular physiological mechanisms of survival and growth of ectothermic vertebrates in low-temperature environments.

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POST-GENOMIC AND DISCOVERY-DRIVEN APPROACHES TO ABIOTIC ENVIRONMENTAL STRESS ADAPTATION IN FISH

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Abiotic environmental fluctuations of temperature, oxygen, ecotoxicants etc present particular challenges to aquatic animals, yet our understanding of the underpinning mechanisms is incomplete and fragmentary. We have adopted a post-genomics, open-screening approach to understand better the nature of effects of environmental stress upon fish, and also to identify the underlying adaptive responses which mitigate these effects. We first developed a large-scale cDNA microarray containing ~13,500 carp-specific DNA probes each of which was sequenced, identified by homology searching and then functionally annotated using a gene ontology (GO) nomenclature. Whilst this approach is technically sophisticated and requires substantial funding to create the necessary resources, new techniques and the increasing availability of sequence data in databases make this much less constrained by funding, and we have recently constructed microarrays for a range of teleost species. We have also developed the informatic pipelines for sequence analysis and functional annotation of probes, the statistical analysis of large array experiments and ontological definition of lists of responding gene using unbiased techniques. In our first major experiment, a screen of 7 tissues from animals subjected to cold exposure has generated lists of ~3000 responding genes, ~1700 of which were identified from homology searching. These were organised into 24 clusters displaying highly tissue-specific responses, and characterisation of these has defined metabolic pathways that are coherently regulated by cold in all major organs. These lists contain several new candidate genes and gene families for subsequent functional analysis that is underway. A second, larger experiment was focused on hypoxia exposure, and we have processed ~600 arrays from 5 different tissues. This has similarly identified suites of gene responses, including a surprising expression of myoglobin in non-muscle tissues such as liver, gill, brain and intestine. The expression of the corresponding gene product has been confirmed by proteomic determination, and the myoglobin has been immunohistologically localised to specific cells in these tissues. A third microarray experiment using trout has focused on the understanding of behavioural phenotypes, comparing the expression of brain genes in fish that have defined dominant or sub-dominant status within a single dominance hierarchy. This experiment used a limited number of brain genes yet identified 5 genes that displayed highly reproducible differences between dominance groups. We confirmed that one of these corresponded to changed levels of protein, and immunological manipulation of the proteins levels in the brain of zebrafish led to distinctive changes in aggressiveness and dominance properties of individuals. Thus these methods of genome-wide analysis are highly flexible and increasingly powerful, generating mechanistic insights into a range of interesting contrasts through the identification of genes and gene networks that respond to experimental manipulation (Funded by NERC-UK).

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THE ROLE OF LEPTIN AND NEUROPEPTIDES IN THE REGULATION OF ENERGY BALANCE DURING LACTATION IN BRANDT'S VOLES (*LASIPODOMYS BRANDTII*)

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Lactation animals match the high energetic cost by increasing the food intake, mobilizing fat and decreasing the thermogenesis in brown fat. The dramatic metabolic and behavioral changes during lactation are accompanied with lower serum leptin level and changes in hypothalamic neuropeptides expression. The mechanisms responsible for the regulation of energy balance during lactation are still not clear. The aim of the present study was to investigate the possible roles of serum leptin and hypothalamic expression of neuropeptide Y (NPY), Agouti-related protein (AgRP), Proopiomelanocortin (POMC) and Cocaine and amphetamine regulated transcript (CART) in Brandt's voles (*Lasiopodomys brandtii*). Female voles were infused recombinant murine leptin with different dosages (0.1, 0.2, 0.4 and 0.6 $\mu\text{g day}^{-1} \text{g}^{-1}$) or PBS (control group) via Alzet mini-osmotic pump during day 10–17 of postpartum. Voles with infusion of leptin significantly increased serum leptin level except at the dosage of 0.1 $\mu\text{g day}^{-1} \text{g}^{-1}$. Leptin infusion can cause significantly reduction in NPY ($P < 0.05$) and AgRP ($P < 0.05$) mRNA expression and increase in POMC mRNA expression ($P < 0.05$) in the higher dosage groups, while CART expression was similar in all groups ($P > 0.05$). Compared with control, food intake significantly decreased ($P < 0.05$) in 0.6 $\mu\text{g day}^{-1} \text{g}^{-1}$ dosage group and showed the decreasing trend in other groups. Leptin infusion did not induce voles to mobilize the maternal body fat further and the litter weight gain which represents the reproduction output was unaffected. However, the maternal body mass decreased in leptin infusion group (decreased 6.2–7.8 g, $P < 0.01$). Decreased maternal body mass can reduce the total energy requirement and favor the growth and survival for pups. These results show that the serum leptin plays important roles in the regulation of energy balance and the feeding behavior during lactation by altering NPY, AgRP and POMC expression in hypothalamus in Brandt's voles.

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GLUCOCORTICOIDS AS SCULPTORS OF ADAPTATION

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Hans Selye coined the terms glucocorticoids (GC), stressor and stress to describe, respectively, the characteristics of adrenal steroid hormones, stimuli and responses that occur when organisms are challenged. In the periphery, stressors, through the actions of GC, mobilize energy stores from muscle and fat and increase gluconeogenic capacity of the liver, and also increase circulating insulin, thus providing adequate glucose for use by brain and red blood cells, while freeing free fatty acids for use by muscle. Provision of adequate energy sources is only one part of the role of the GC, however. Once provided, the energy must be used through changes in behavior that are also mediated by stressor-induced secretion of these protean hormones. GC feed forward on the brain to affect not only learning and memory, but also to enhance the intensity of behaviors that are determined by the immediate context. Thus, if male rats are in a social dominance–subordination situation, the steroids increase the level of aggression observed; if wheels are available, rats run more with high GC, and, if palatable food is available, they eat more. These responses are in all likelihood a consequence of the direct actions of the GC on monoaminergic