METABOLIC AND MOLECULAR RESPONSES OF FISH TO HYPOXIA

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Hypoxia survival requires a well-coordinated response to either secure more O₂ from the depleted environment or to defend against the metabolic consequences of too little O₂ at the mitochondria, which limits aerobic ATP production. Inhibition of aerobic ATP production during hypoxia exposure imposes a substrate-limited cap on the duration of survival because O₂-independent ATP production (anaerobic) is far less efficient than aerobic ATP production. It has long been held that hypoxia-tolerant animals are able to extend the period of survival under severely hypoxic conditions through a depression of basal metabolic rate, which limits the extent of activation of O₂-independent pathways of ATP production. This contention appears to be supported by the available literature; however, more studies measuring metabolic rate during hypoxia exposure are needed before a definitive outcome can be decided. Duration of hypoxia exposure is also an important component to consider when assessing the responses to hypoxia. Long-term hypoxia exposure (> a few hours in some cases) can result in large changes in
gene expression, which underlie acclimation/acclimatization and potentially enhance hypoxic survival. Hypoxia-mediated changes in gene expression are likely regulated by the transcription factor, hypoxia inducible factor (HIF), which is well characterized in mammalian systems, but has only recently been examined in fish. Hypoxia inducible factor appears to be regulated in a similar fashion in fish as in mammals, but to date, there does not appear to be a direct link between HIF function and hypoxia tolerance in fish.

1. INTRODUCTION

Environmental hypoxia is a common, naturally occurring phenomenon in many aquatic ecosystems, the prevalence of which is increasing due to anthropogenic nutrient loading and eutrophication (reviewed in Chapter 1). In light of these O₂ fluctuations in the aquatic environment, it is perhaps not surprising that among all vertebrates, fish boast the largest number of hypoxia-tolerant species; hypoxia has clearly played an important role shaping the evolution of many unique adaptive strategies for hypoxic survival. Previous chapters in this volume have outlined a myriad of physiological and biochemical strategies that facilitate O₂ uptake under hypoxic conditions including changes in behavior, ventilation, hemoglobin-O₂ binding characteristics, and cardiovascular function. These strategies work to sustain metabolic function by maximizing O₂ extraction from the environment. Of importance to the present chapter, however, are the biochemical and molecular strategies that are responsible for defending against the metabolic consequences of O₂ levels that fall below a threshold where metabolic function is affected or cannot be maintained. Paramount to this defense strategy is a well coordinated response to maintain cellular ATP turnover, albeit at reduced levels, and the ability for hypoxic acclimation to “enhance” cellular and whole animal function under O₂ limiting conditions.

Metabolic and molecular responses to hypoxia are critical to enhance survival at O₂ levels below a species critical oxygen tension (Pₐₘ). In the context of this chapter, Pₐₘ is defined as the environmental O₂ tension at which an organism’s O₂ consumption rate transitions from being independent of environmental O₂ to being dependent on environmental O₂ (see Figure 10.1A; Pörtner & Grieshaber 1993). As such, Pₐₘ represents a whole-animal measure of O₂ extraction capacity from the environment and is considered by many researchers as an indicator of hypoxia tolerance (Chapman et al., 2002). Many physiological adjustments can affect Pₐₘ, and the majority of these have been outlined in previous chapters in this volume. For example, increases in O₂ extraction capacity through modifications to ventilation (see Chapter 5), O₂ transport systems (see Chapter 6),
or O₂ delivery systems (see Chapter 7) can theoretically result in a decrease in P_{crit}, and thus an enhancement of hypoxia tolerance. In contrast, increases in whole animal metabolic demands associated with, for example, gonad development and reproduction (see Chapter 3) as well as during digestion

Fig. 10.1. Metabolic responses of fish to changes in environmental O₂. (A) A species’ critical oxygen tension (P_{crit}) is the point at which O₂ consumption rate transitions from being independent of environmental O₂ levels (often referred to as O₂ regulation) to being dependent on environmental O₂ (often referred to as O₂ conforming). P_{crit} can be increased, detrimentally affecting hypoxia tolerance by increasing energetically expensive processes such as reproduction, growth, or digestion. P_{crit} can also be decreased, enhancing hypoxia tolerance through changes in respiration (V_{E} and gill perfusion), O₂ transport/delivery (changes in hemoglobin-O₂ binding affinity and cardiovascular responses), or through reductions in energetically expensive processes such as reproduction, digestion, and swimming. (B) At O₂ levels below P_{crit}, survival is dependent upon the ability of an animal to suppress basal metabolic rate to limit the extent of the activation of O₂-independent pathways of ATP production. See text for more detail.
and allocation of energy to growth (see Chapter 8) can cause an increase in $P_{\text{crit}}$, and a decrease in hypoxia tolerance. Thus, suppression of reproduction, digestion, and growth during hypoxia exposure reduces metabolic demands and enhances hypoxia tolerance and survival (Figure 10.1A).

2. THE METABOLIC CHALLENGE OF HYPOXIA EXPOSURE

At $O_2$ levels below $P_{\text{crit}}$, the fundamental challenge is one of metabolic energy balance. Greater than 95% of the $O_2$ consumed by a fish in normoxia is used as the terminal electron acceptor by the mitochondrial electron transport chain for ATP production (via oxidative phosphorylation). If environmental hypoxia leads to hypoxemia (i.e., physiological mechanisms to enhance $O_2$ uptake are insufficient to protect the animal from its environment and blood $O_2$ content is reduced), then there is the potential for an $O_2$ limitation at the mitochondrion, which imposes limitations on the capacity for ATP production. Under these conditions, ATP can only be generated by processes such as glycolysis yielding lactate production or through direct phosphate transfer from phosphorylated intermediates such as creatine phosphate (CrP). These processes of direct phosphate transfer from a substrate to ADP forming ATP are termed substrate-level phosphorylation. Although these processes of ATP generation can occur during periods of $O_2$ lack, the amount of ATP produced per mole of substrate consumed is approximately 15- to 30-fold lower than if mitochondrial respiration occurs. For example, aerobic catabolism of 1 mole of glucose yields ~30 moles of ATP, while the anaerobic catabolism of glucose, involving only glycolysis and lactate production, produces 2 moles of ATP. A reduction in the ability of an organism or cell to generate sufficient ATP to meet metabolic demands presents a problem for the maintenance of cellular energy balance. Hypoxia-sensitive animals quickly succumb to hypoxia due to an inability to maintain cellular energy balance and a loss of cellular $[\text{ATP}]$ (Boutilier, 2001). Thus, during hypoxia the inhibition of $O_2$-based mitochondrial ATP production imposes a potential substrate-limited cap on the duration of survival. Under these $O_2$ limiting conditions duration of survival is dictated by two, interrelated factors: (1) the ability to reduce metabolic demands through a controlled metabolic rate suppression; and (2) the availability of substrate for $O_2$-independent ATP production. Illustrated in Figure 10.1B is a conceptual framework to understand the relationship between metabolic rate suppression and capacity for $O_2$-independent ATP production. At environmental $O_2$ tensions below $P_{\text{crit}}$, hypoxia tolerance is likely to be dictated by the degree of metabolic rate suppression, which extends the length of time a fixed quantity of fermentable substrate can support cellular function. For example, scenario 1 would
represent a severely hypoxia-sensitive fish, where at O$_2$ tensions below $P_{crit}$, the animal attempts to maintain metabolic rate, which can only be accomplished by a large activation of O$_2$-independent pathways of ATP production (largely glycolysis) thus utilizing fermentable fuels at a high rate (indicated by the large curved arrow). If the quantity of fermentable fuels is limited, then the animal will quickly succumb to hypoxia and die. On the other hand, scenarios 2 and 3 represent increasing levels of hypoxia tolerance, where decreases in metabolic rate limit the magnitude of the activation of O$_2$-independent ATP production (shorter curved arrows) and extend the period of time that can be supported by substrate-level phosphorylation. Thus, it seems reasonable to hypothesize that there should be a relationship between hypoxia tolerance, the magnitude of the hypoxia-induced metabolic rate suppression, and the availability of fermentable fuels to support O$_2$-independent ATP production.

At the cellular level, the precise mechanism of hypoxia-induced death is not known; however, it is clear that hypoxic death in fish is associated with catastrophic loss of substrate, failure of essential ATP consuming processes, accumulation of toxic levels of waste products (protons and lactate), and cellular necrosis. Underlying all of these potential causes of hypoxia-induced death is an inability of the animal to maintain metabolic energy balance. Boutilier and St-Pierre (2000) analyzed the available literature and proposed an elegant hypoxia-induced (and hypothermia-induced) cascade of events that yield necrotic cell death. In hypoxia-sensitive animals, hypoxia exposure leads to an inability to generate sufficient ATP to meet the metabolic demands of cellular ion regulation, protein synthesis, and other metabolic processes – a mismatch between ATP supply and demand – therefore, cellular [ATP] falls to levels that are insufficient to maintain the activity of these energy-consuming processes. Boutilier and St-Pierre (2000) pointed to cellular ion regulation to be the most critical aspect of cell survival and proposed that a loss of ATP limits the capacity of a cell to maintain transmembrane potential resulting from net Na$^+$ influx and K$^+$ efflux. This results in depolarization of plasma and organelle membranes, Ca$^{2+}$ accumulation in the cytosol from organelles and extracellular fluid, the activation of phospholipases and Ca$^{2+}$-dependent proteases, and the rupture of membranes, ultimately resulting in necrotic cell death. It has been proposed that hypoxia-tolerant animals are able to stave off these catastrophic events by initiating regulated metabolic rate suppression and stabilizing cellular [ATP].

Stable cellular [ATP] during hypoxia exposure is often accepted as the hallmark measure of a hypoxia-tolerant animal (Hochachka et al., 1996; Boutilier, 2001); however, this has been demonstrated to be an over simplification. Numerous studies have shown a substantial disruption of cellular energetics during hypoxia exposure even in hypoxia-tolerant organisms (van den Thillart et al., 1980,1989; Borger et al., 1998; Hallman et al., 2008;
and changes in cellular [ATP] appear to be tissue specific. For example, in muscle, numerous studies have demonstrated that cellular [ATP] is not affected by hypoxia/anoxia exposure (van den Thillart et al., 1980; Richards et al., 2007, 2008); while in liver, [ATP] decreases initially upon hypoxia exposure and then stabilizes at a lower concentration (Figure 10.2A) (J. Dalla Via et al., 1994; Jibb and Richards, 2008; van den Thillart et al., 1980). These results are in general agreement with the results of Busk and Boutilier (2005) who showed in isolated eel hepatocytes that anoxia exposure caused an initial decrease in [ATP], followed by a stabilization at a new, lower level. In contrast, Krumschnabel et al. (1997) demonstrated that exposure of isolated goldfish hepatocytes to anoxia did not result in a decrease in [ATP], while the same preparation exposed to chemical anoxia, using NaCN, showed a decrease in [ATP]. This latter decrease in [ATP] was modest when compared with the large decreases of [ATP] observed in anoxia-exposed hepatocytes isolated from the hypoxia-intolerant rainbow trout (Krumschnabel et al., 1997). It has been postulated that the reason for the differences in response in [ATP] between muscle and liver is related to the tissue [CrP]. Muscle [CrP] are much higher than measured in liver (20 to 50 versus <5 µmol/g wet tissue, respectively); thus, in liver, there is a lack of capacity to buffer [ATP] during the onset of hypoxia.

Whether tissue [ATP] is affected by hypoxia or not, intracellular acidosis and CrP hydrolysis result in an accumulation of [ADPfree] and [AMPfree], causing increases in [ADPfree]/[ATP] and [AMPfree]/[ATP] and substantial losses of cellular phosphorylation potential (Figure 10.2; Hallman et al., 2008; Jibb and Richards, 2008; Richards et al., 2008; van den Thillart et al., 1989). This disruption of cellular energy status plays several important roles in the cell during hypoxia exposure. First, decreases in phosphorylation potential may affect rates of cellular ATP production and substrate oxidation. For example, hypoxia exposure was associated with a significant drop in the free energy of ATP hydrolysis (ΔG'; Figure 10.2C) (Hallman et al., 2008; Jibb and Richards, 2008; Richards et al., 2008). Estimates of the critical limit of ΔG' for the maintenance of cellular function suggest that below a threshold of −52 kJ/mol, cellular processes such as ion pumping can no longer derive sufficient energy from ATP hydrolysis to be maintained (Hardewig et al., 1998; Jansen et al., 2003). Second, changes in cellular [ADPfree]/[ATP] and [AMPfree]/[ATP] are important signals coordinating the metabolic responses to hypoxia. For example, increases in [ADPfree]/[ATP] are known to allosterically activate glycolysis, increasing O2-independent ATP production and more recent evidence indicates that increases in [AMPfree]/[ATP] may be vital to overall coordination of metabolic rate suppression in certain tissues of hypoxia-tolerant fish (Jibb and Richards, 2008).
Fig. 10.2. Liver [ATP] (A), calculated [ADPfree]/[ATP] (B), Gibbs free energy of ATP hydrolysis (C) ($\Delta G'_{\text{ATP}}$) in goldfish exposed to normoxia and 12 h of hypoxia (<0.5% air saturation). Horizontal dashed lines through normoxia are shown as a reference. [Data from Jibb and Richards (2008) with permission.]
3. THE CONCEPT OF TIME IN THE METABOLIC RESPONSES TO HYPOXIA

When considering the physiological and biochemical responses to hypoxia, environmental O₂ levels are not the only factor to consider: the length of time spent in hypoxia can have dramatic effects on the responses to O₂ lack. Upon exposure to hypoxia, immediate survival is dependent upon the ability of the fish to quickly modify existing physiological and biochemical systems in an attempt to maintain metabolic function. If these immediate responses are sufficient for survival of the onset of hypoxia, then an animal has the opportunity to acclimate or acclimatize, which, for the most part, is thought to be of benefit in enhancing the ability of an animal to survive hypoxic exposure. At the heart of any acclimation response are changes in gene expression, which can alter the capacity of an animal to endure hypoxia. Changes in gene expression, if they are translated into functional changes in protein amount or possibly protein turnover rates, can affect hypoxia survival by either increasing or decreasing the amounts of specific proteins in a metabolic pathway. For example, large increases in the expression of the lactate dehydrogenase gene (ldh) and increases in LDH activity have been observed during both long- and short-term hypoxia exposure in fishes (e.g., Amazonia cichlid) (Almeida-Val et al., 1995, 2006). In addition, selective changes in gene expression can result in protein isoform switching, which in some cases has been shown to enhance survival to environmental perturbation (Schulte, 2004). Environmental hypoxia is well known to affect gene expression patterns in fish with several microarray studies showing changes in the transcription of genes involved in O₂ uptake, energy turnover, growth and development, immune responses, cell signaling, and stress (Figure 10.3 and Table 10.1; Gracey et al., 2001; Ton et al., 2003). Thus, almost every physiological and biochemical response discussed earlier in this volume is regulated, at least in part, by changes in gene expression. Regulation and coordination of changes in gene expression in response to hypoxia exposure are mediated largely by the transcription factor hypoxia inducible factor (HIF), which has been characterized in mammalian and fish systems.

The remaining portion of this chapter is divided into two parts. The first section outlines the metabolic and molecular responses of fish to hypoxia exposure. Combining the metabolic and molecular gene expression changes is meant to emphasize that the changes observed in metabolic phenotype are also controlled to a degree by changes in gene expression, which underlie acclimation responses. The second portion of this chapter examines how these processes are coordinated at the biochemical and molecular level with emphasis on HIF as a regulator of hypoxia-induced changes in gene expression.
Many excellent reviews have summarized the metabolic and molecular responses of fish and other lower vertebrates to hypoxia exposure (Almeida-Val et al., 2006; Bickler and Buck, 2007; Nikinmaa and Rees, 2005). Many of the chapters in this book outline numerous responses to hypoxia including responses that work to increase O_2_ uptake and the metabolic adjustments.
Table 10.1
Molecular responses to hypoxia

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Temp. (°C)</th>
<th>Hypoxia</th>
<th>Duration</th>
<th>Whole animal or tissue</th>
<th>Gene expression changes</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longjaw mudsucker</td>
<td><em>Gillichthys mirabilis</em></td>
<td>15</td>
<td>10% air sat.$^a$</td>
<td>8, 24, 72, and 144 h</td>
<td>Liver</td>
<td>↑ glycolysis (7)</td>
<td>Gracey et al. (2001)</td>
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<td></td>
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<td></td>
<td></td>
<td>↑ amino acid metabolism</td>
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<td></td>
<td></td>
<td>↑ iron and Hb metabolism (8)</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>↑ anti-growth &amp; cell proliferation (10)</td>
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<td></td>
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<td>↑ aerobic metabolism (4)</td>
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<td>Muscle</td>
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<td>↑ glycolysis (4)</td>
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<td>↓ glycolysis &amp; CK (5)</td>
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<td>↓ aerobic metabolism (4)</td>
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<td>↓ locomotion and contraction (9)</td>
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<td></td>
<td></td>
<td>↓ protein synthesis (15)</td>
<td></td>
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<tr>
<td>Zebrafish</td>
<td><em>Danio rerio</em></td>
<td>28</td>
<td>5% oxygen 23% air sat.</td>
<td>24 h</td>
<td>48 h post-fertilization embryos</td>
<td>↑ glycolysis (6)</td>
<td>Ton et al. (2003)</td>
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<td></td>
<td>↓ cell signaling (5)</td>
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<td></td>
<td></td>
<td>↓ aerobic metabolism (10)</td>
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<td></td>
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<td>↓ creatine kinase (2)</td>
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<td>↓ cell structure &amp; mobility (20)</td>
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<td>↓ ion transporting ATPases (5)</td>
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<td></td>
<td></td>
<td>↓ protein synthesis (6)</td>
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<td>↓ iron &amp; Hb metabolism (5)</td>
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<td></td>
<td></td>
<td>↓ cell division (5)</td>
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<td></td>
<td></td>
<td></td>
<td>↓ cell/organism defense (5)</td>
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</table>

$^a$ air sat. = air saturation.
Zebrafish *Danio rerio*  28  10% air sat.  21 days  
Gills  

↑ glycolysis (2)  
↑ disease defense (12)  
↑ phosphatases (6)  
↑ chaperones (8)  
↓ aerobic metabolism (31)  
↓ protein synthesis (54)  
↑ elongation factors (2)  
↓ stress response (6)  
↓ apoptosis (8)  
↓ locomotion and contraction  
↓ growth regulation (12)  
↓ innume response (9)  
↓ proteosome degradation (6)  

van der Meer *et al.* (2005)

The numbers of genes indicated may have either increased or decreased in expression, but overall the authors concluded that the changes indicated would yield an overall change in biological outcome indicated by the arrow.

*P*$_{\text{crit}}$ of this species is 1.2 mg O$_2$/L and hypoxia exposure was 0.8 mg O$_2$/L

*Decrease in oxygen occurred gradually over 4 days.*
associated with hypoxia exposure in the heart (see Chapter 7) and the metabolic aspects yielding the extraordinarily anoxia-tolerant crucian carp (*Carassius carassius*; see Chapter 9). From all of the preceding chapters it has become evident that there are three principal aspects that function to maintain cellular energy balance and these include: (1) increased O$_2$ uptake from the hypoxic environment to sustain a modicum of aerobic ATP production; (2) strong activation of an O$_2$-independent means of ATP production; and (3) a reduction in metabolic demands through regulated metabolic rate suppression, which is described in more detail below.

4.1. Increases in O$_2$ Transport

As outlined previously in this volume, the physiological and biochemical responses that yield increases in O$_2$ transport capacity are important adaptations to survive hypoxia. Indeed, recent work by Mandic *et al.* (2008) showed in a group of closely related intertidal fish species (sculpins from the family Cottidae) that approximately 75% of the variation in hypoxia tolerance (assessed as P$_{crit}$) could be explained by variation in physiological attributes affecting O$_2$ uptake (Hb–O$_2$ binding affinity or gill surface area) or O$_2$ use (routine metabolic rate). However, since other chapters have explicitly dealt with the physiological responses that increase O$_2$ uptake from the environment and O$_2$ delivery to the tissues, in this section I will solely focus on the O$_2$-dependent changes in gene expression that may form the foundation of possible acclimation responses. In fact, almost every microarray study performed to date has shown an effect of hypoxia exposure on mRNA levels for proteins involved in Hb metabolism and oxygen transport (Figure 10.3 and Table 10.1).

As pointed out in Chapter 6, modifications to Hb–O$_2$ binding affinity and blood Hb content are important responses to hypoxia. At the gene expression level, Gracey *et al.* (2001) showed dramatic changes in the expression of genes involved in heme metabolism in liver of the mudsucker in response to hypoxia exposure. Several genes involved in iron-heme catabolism and heme protein turnover were all induced by hypoxia exposure. These general changes in genes involved in iron and heme metabolism could be linked with hypoxia-induced erythropoietin (EPO) or erythropoiesis and increased demand for iron from hemoglobin synthesis. By contrast, zebrafish embryos exposed to hypoxia show a general decrease in the expression of genes involved in Hb metabolism. Specifically, Ton *et al.* (2003) showed large decreases in mRNA levels for globin, $\beta$A1, hemoglobin $\beta$ chain, globin $\alpha$-embryonic, globin 2 $\alpha$-embryonic, and, oddly, erythropoietin. The probable explanation for these counterintuitive decreases in mRNA levels for proteins involved in blood O$_2$ transport is that the very small zebrafish embryos do not require blood flow for survival and O$_2$ uptake is mostly via diffusion.
In mammals, hypoxia is a powerful regulator of the production of erythropoietin (Semenza and Wang, 1992), which causes an increase in red blood cell production leading to increases in Ht and increases in blood O2-carrying capacity. There is no published data directly linking hypoxia exposure and changes in EPO gene expression in fish, although injection of human EPO into goldfish stimulates red blood cell production (Taglialatela and Della Corte, 1997) demonstrating that if EPO is synthesized it could enhance red blood cell production. It must be pointed out, however, that the available data on hypoxia-induced EPO regulation in fish is not clear in its conclusion. When Fugu EPO gene and promoter region constructs (6 kb) are transfected into human carcinoma cell lines transcription is not hypoxia responsive (Chou et al., 2004) and this is supported by a lack of an HIF-binding hypoxia response element (HRE) in the promoter region of Fugu. However, when these same promoter region constructs were transfected into fish cell lines, increased expression of an alternatively spliced EPO transcript was observed in cells subjected to hypoxia (Fraser et al., 2006), suggesting at least some degree of hypoxia regulation of EPO in fish.

Hypoxia-induced changes in myoglobin (Mb) expression have recently received considerable attention in fish and have been discussed by Wells (see Chapter 6) and Gamperl and Driedzic (see Chapter 7). Typically, Mb is expressed at high levels in red-skeletal and cardiac muscle, but recent evidence has shown a hypoxia-induced expression of Mb in nonmuscle tissues of the hypoxia-tolerant common carp (Cyprinus carpio; Fraser et al., 2006) and in the gills of zebrafish (van der Meer et al., 2005). In the carp, increases in Mb mRNA were observed during 1–8 days of hypoxia exposure in the liver, gill, and brain. Increases in mRNA were reflected in increased protein expression determined using 2D gel electrophoresis, which suggests that the increase in Mb expression may enhance O2 diffusion into tissues during hypoxia exposure. Enhanced expression of Mb in the gills of zebrafish (van der Meer et al., 2005), suggests a potential generalized role for Mb in facilitating O2 transport in fish tissues; however, it is interesting to reiterate that the expression of Hb genes were not affected in the same gills during hypoxia exposure. A brain-specific myoglobin was also identified in the common carp, distinct from neuroglobin, but it was not hypoxia responsive at the transcript level. Additional details on Mb and neuroglobin expression in fish can be found in Chapter 6 of this volume.

4.2. O2-Independent ATP Production

Hypoxia exposure in fish elicits a strong activation of substrate-level phosphorylation via glycolysis and CrP hydrolysis and a decrease in aerobic metabolism. Endogenous glycogen typically serves as the carbohydrate store
for glycolysis, thus the levels of tissue glycogen are indicative of the capacity of a tissue to support ATP turnover via glycolysis. Furthermore, due to the suppression of appetite and digestive function during hypoxia (see Chapter 8) endogenous stores of fermentable fuels represent the only source of substrate to support ATP production. As illustrated in Table 10.2, hypoxia-tolerant animals such as carp, goldfish, killifish, and oscar typically have higher levels of tissue glycogen relative to animals considered to be hypoxia sensitive (e.g., rainbow trout). Thus, it seems reasonable to conclude that across broad taxonomic groups of fish, those animals with more glycogen will be able to produce more ATP for longer periods of time at lower O2 levels (see Figure 10.1B). Another striking feature illustrated by Table 10.2 is the very large glycogen stores that occur in liver compared with those observed in other tissues including the heart, brain, and skeletal muscle. Liver glycogen is thought to serve as a repository of glucose that can be used by other tissues to support glycolytic ATP production during hypoxia exposure; however, for this to occur the glucose liberated from liver glycogen must be transported between tissues. The details of glucose transport during hypoxia exposure are outlined in Chapter 7.

At the molecular level, every cDNA microarray study performed on fish has shown a typical hypoxia-induced metabolic switch, that is, a reduction in mRNA levels for proteins involved in aerobic metabolism and an increase in the mRNA levels for proteins involved in anaerobic metabolism (see Table 10.1; glycolysis, creatine kinase, and aerobic metabolism). For example, in zebrafish embryos, Ton et al. (2003) showed a decrease in the expression of mRNA coding for genes involved in the TCA cycle, including succinate dehydrogenase, malate dehydrogenase, and citrate synthase, and an increase in expression of genes involved in glycolysis including phosphoglycerate mutase, phosphoglycerate kinase, enolase, aldolase, and lactate dehydrogenase (details of hypoxia exposure given in Table 10.1). Similarly, in gills of zebrafish exposed to hypoxia, the levels of mRNA for proteins involved in the TCA cycle and electron transfer chain were all decreased signifying an overall decline in mitochondrial ATP production (van der Meer et al., 2005). Simultaneously, increases in mRNA coding for proteins involved in glycolytic ATP production were noted in the gill during hypoxia exposure, including increases in glycogen phosphorylase and aldolase. Further, there was a general decrease in the expression of genes that code for proteins involved in fat metabolism, cellular uptake, and transport, including acyl-CoA dehydrogenase, intestinal fatty acid binding protein, and other metabolite binding proteins. Also associated with the metabolic switch from aerobic to anaerobic metabolism were highly sensitive changes in the expression of pyruvate dehydrogenase kinase, which was up-regulated in muscle of killifish (Fundulus heteroclitus) during hypoxia exposure, but
Table 10.2
Glycogen content in brain, liver, and muscle of fish

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Brain</th>
<th>Liver</th>
<th>Skeletal muscle</th>
<th>Heart</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crucian carp</td>
<td><em>Carassius carassius</em></td>
<td>13 to 204</td>
<td>123 to 2160</td>
<td></td>
<td>18 to 493</td>
<td>Voranen <em>et al.</em> (Chapter 9)</td>
</tr>
<tr>
<td>Goldfish</td>
<td><em>Carassius auratus</em></td>
<td>13 to 20</td>
<td>800</td>
<td>30</td>
<td>142</td>
<td>Voranen <em>et al.</em> (Chapter 9); Mandic <em>et al.</em> (In Press)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td><em>Oncorhynchus mykiss</em></td>
<td>0.5</td>
<td>110</td>
<td></td>
<td>25 to 60</td>
<td>Voranen <em>et al.</em> (Chapter 9)</td>
</tr>
<tr>
<td>Killifish</td>
<td><em>Fundulus heteroclitus</em></td>
<td>N/A</td>
<td>299 to 550</td>
<td>10 to 40</td>
<td>N/A</td>
<td>Fangue <em>et al.</em> (2008); Richards <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>Oscar</td>
<td><em>Astronotus ocellatus</em></td>
<td>203 to 279</td>
<td>25 to 30</td>
<td></td>
<td></td>
<td>Chippari-Gomes <em>et al.</em> (2005); Richards <em>et al.</em> (2007)</td>
</tr>
<tr>
<td>Blue discus</td>
<td><em>Symphysodon aequifasciatus</em></td>
<td>100</td>
<td>15</td>
<td></td>
<td></td>
<td>Chippari-Gomes <em>et al.</em> (2005)</td>
</tr>
<tr>
<td>Tilapia</td>
<td><em>Oreochromis mossambicus</em></td>
<td></td>
<td>175 mg/g protein</td>
<td></td>
<td></td>
<td>Chang <em>et al.</em> (2007)</td>
</tr>
<tr>
<td>African Lungfish</td>
<td><em>Protopterus dolloi</em></td>
<td>98 to 180</td>
<td>8 to 10</td>
<td></td>
<td>50 to 60</td>
<td>Frick <em>et al.</em> (2008)</td>
</tr>
<tr>
<td>Pacu</td>
<td><em>Piaractus mesopoamicus</em></td>
<td>ca. 500</td>
<td>15</td>
<td></td>
<td></td>
<td>Moraes <em>et al.</em> (2006)</td>
</tr>
<tr>
<td>Silver catfish</td>
<td><em>Rhamdia quelen</em></td>
<td></td>
<td></td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Glycogen content is reported in μmol glucosyl units/g wet tissue unless otherwise stated.
these changes did not yield measurable changes in PDK protein content (Richards et al., 2008).

Associated with the large increases in the expression of glycolytic enzymes, increases in mRNA levels for proteins involved in amino acid catabolism have been demonstrated. In the liver of longjaw mudsucker, Gracey et al. (2001) noted increases in $S$-adenosylmethionine synthase and cystathionine synthase, which catalyze steps in methionine degradation as well as several aminotransferases. Consistent with the induction of aminotransferases was the coexpression of glutamine synthetase, which catalyzes the major liver ammonia detoxification reaction of the synthesis of glutamine from glutamate. Catabolism of gluconeogenic amino acids, such as tyrosine and serine, yields either pyruvate or TCA cycle intermediates, both of which can serve as carbon skeletons for gluconeogenesis. Further evidence linking amino acid catabolism with hypoxia-induced gluconeogenesis is that the expression of glucose-6 phosphatase was strongly induced in response to hypoxia. Glucose-6 phosphatase catalyzes the dephosphorylation of glucose-6 phosphate to glucose, which can be transported in the circulation to other tissues to fuel glycolysis. Thus, for the longjaw mudsucker, amino acid catabolism coupled with gluconeogenesis in the liver may represent a mechanism to maintain blood glucose levels during hypoxia and may contribute to maintaining whole animal energy balance.

Changes in the mRNA levels for several metabolite transporters have also been noted in many studies. For example, mRNA for MCT4, a membrane-bound lactate/pyruvate transporter, increased in response to hypoxia exposure in zebrafish (Ton et al., 2003). Furthermore, there were dramatic increases in the expression of glucose transporters (GLUT) in eye, gill, and kidney of grass carp during exposure to hypoxia (up to 170 h at ~0.6% air saturation; Zhang et al., 2003). Changes in both of these transporters in response to hypoxia exposure indicate an overall increase in the movement of substrates for glycolysis and waste products (lactate).

Tissue-specific effects of hypoxia exposure have been noted in several studies suggesting that not all tissues respond similarly to hypoxia. Differential gene expression responses have been noted in the liver and muscle of the longjaw mudsucker during hypoxia exposure (Gracey et al., 2001). In the liver of the mudsucker, there was an overall increase in mRNA levels for proteins involved in glycolysis, with large increases observed in mRNA for LDH-A, triosephosphate isomerase, PFK-2/FBP-2, enolase, and glucose 6-phosphatase. Smaller, yet significant increases in mRNA were also noted for cytochrome $b$ and cytochrome $c$ oxidase, which are proteins of the mitochondrial electron transport chain, and possibly point to an enhancement of overall capacity for the mitochondria in liver to sustain at least some level of ATP production. In muscle tissue, however only minor increases in
mRNA for the glycolytic enzymes (LDH-A and PFK-2) were observed and in direct contrast to the response observed in liver, substantial decreases in mRNA levels for the glycolytic enzymes enolase, GAPDH, and glucose 6-phosphate dehydrogenase, as well as creatine kinase were observed. Furthermore, unlike the effects of hypoxia on liver mRNA levels, there were substantial increases in the expression of cytochrome b and cytochrome c oxidase I in muscle.

In general, the tissue-specific responses observed in the mudsucker at the mRNA level is consistent with the tissue-specific effects of 4 weeks of hypoxia exposure (~15% air saturation) in Fundulus grandis (Martinez et al., 2006). This study clearly demonstrated that enzyme activities of glycolysis and glycogen metabolism were strongly suppressed by hypoxia exposure in skeletal muscle, while in liver there was evidence for increases in several enzymes involved in glycolysis and carbohydrate oxidation (Figure 10.4). Fewer changes in glycolytic and glycogen enzymes were observed in the heart and brain compared with the liver and muscle and those that did change, did so with a smaller magnitude. Interestingly, among the tissues that showed general increases in enzymes within the glycolytic pathway, the enzymes that increased were not always the same enzymes. Martinez et al. (2006) speculated that tissue-specific differences in the responses to long-term hypoxia in Fundulus grandis reflect the balance of energetic demands, metabolic role, and oxygen supply to the tissues. More studies are needed to examine the tissue-specific effects of hypoxia exposure on metabolic energy supply.

4.3. Metabolic Rate Suppression

The ability to suppress cellular ATP demand to match the limited capacity for O2-independent ATP production has emerged as the unifying adaptive strategy ensuring hypoxia survival (Hochachka et al., 1996). Because ATP turnover rates cannot be measured directly in vivo, those interested in measuring metabolic rate must use indirect measures. The two typical indirect measures for metabolic rate is O2 consumption and heat loss. Measurements of O2 consumption only determine the contributions of aerobic metabolism to overall ATP turnover and therefore during periods of metabolic stress that lead to increases in substrate-level phosphorylation O2 consumption can underestimate total ATP turnover or metabolic rate. The best indirect measure of metabolic rate (often referred to as the “direct” measure of metabolic rate to demonstrate its superiority) is the measurement of heat loss. Metabolic heat production is proportional to ATP turnover, therefore a reduction in heat loss can be directly linked with a reduction in total ATP turnover and metabolic rate suppression.
4.3.1. Evidence of Metabolic Rate Suppression in Fish

Heat production in fish during hypoxia/anoxia exposure has been assessed in several species including goldfish (*Carassius auratus*; Stangl & Wegener, 1996; van Waerversveld *et al*., 1988a,b; van Ginneken *et al*., 2004), crucian carp (*Carassius carassius*; Johansson *et al*., 1995), tilapia

Fig. 10.4. Effects of long-term hypoxia exposure on glycolytic enzyme activities (i.u. mg⁻¹ protein) in tissues of *Fundulus grandi*. The y axis represents the percentage change in the mean value for each enzyme measured from hypoxic fish relative to the normoxic value for (A) skeletal muscle, (B) liver, (C) heart, and (D) brain. HK, hexokinase; PGI, phosphoglucomutase; PFK, phosphofructokinase; ALD, aldolase; TPI, triose phosphate isomerase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerokinase; PGM, phosphoglyceromutase; ENO, enolase; PYK, pyruvate kinase; LDH, lactate dehydrogenase. [Data from Martinez *et al*. (2006) with permission.]
(Oreochromis mossambicus; van Ginneken et al., 1997, 1999), European eel (Anquilla anquilla; van Ginneken et al., 2001), zebrafish (Brachydanio rerio; Stangl & Wegener, 1996), and in isolated hepatocytes from rainbow trout (Oncorhynchus mykiss; Rissanen et al., 2006) (see Table 10.3). Interestingly, across a very broad range of fish species including those considered to be hypoxia tolerant (crucian carp, goldfish, and tilapia) and tissues from those considered to be intolerant (rainbow trout), all species show the capacity to decrease metabolic rate in response to hypoxia exposure. The most impressive reductions in metabolic rate, however, still occurred in the goldfish, tilapia, and European eel with an \( \sim 70\% \) decrease in metabolic rate during hypoxia exposure (Table 10.3). Hepatocytes isolated from rainbow trout showed a decreased metabolic rate to a lesser degree than seen in more hypoxia-tolerant animals such as goldfish and tilapia (whole-animal measurements), but comparisons between isolated tissues and whole animals are difficult to make because of tissue-specific responses to hypoxia. Oddly, zebrafish exposed to severe hypoxia (\(<6\%\) air saturation) for only 50 min showed a progressive increase in heat production indicating an overall increase in metabolic rate during hypoxia exposure (Stangl and Wegener, 1996). This increase in metabolic rate may represent increased costs associated with hypoxia-induced movement and escape behavior. Although metabolic rate suppression is clearly a response of fish to hypoxia/anoxia exposure, due to the limited number of studies available it is not possible to comment with any certainty on the direct association between the degree of metabolic rate suppression and overall hypoxia tolerance.

4.3.2. MECHANISMS OF METABOLIC RATE SUPPRESSION

The question of how organisms are able to reduce metabolic rate below routine levels has received considerable attention over the past several decades. Original work in this field using hepatocytes isolated from the anoxia-tolerant turtle (Chrysemys picta), demonstrated that a 94% suppression in metabolic rate during anoxia exposure was achieved through the dramatic down-regulation of Na pumping, protein turnover, urea synthesis and gluconeogensis (Buck & Hochachka, 1993; Buck et al., 1993a,b; Land et al., 1993; Hochachka et al., 1996; Hochachka and Lutz, 2001). It is now clear that cellular mechanisms underlying metabolic rate suppression are similar across broad taxonomic groups with metabolic rate suppression involving the controlled arrest of processes involved in membrane ion movement (Buck and Hochachka, 1993; Richards et al., 2007), protein synthesis (Lewis et al., 2007; Wieser and Krumbschnabel, 2001), RNA transcription, urea synthesis, gluconeogensis, and other anabolic pathways (Hochachka et al., 1996).
<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Whole animal or tissue</th>
<th>Temperature (°C)</th>
<th>Hypoxia</th>
<th>Duration</th>
<th>Metabolic Rate Suppression (% decrease from normoxia)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldfish</td>
<td><em>Carassius auratus</em></td>
<td>Whole animal</td>
<td>20</td>
<td>Anoxia</td>
<td>2 to 3h</td>
<td>70</td>
<td>van Waversveld <em>et al.</em> (1988, 1989a)</td>
</tr>
<tr>
<td>Goldfish</td>
<td><em>C. auratus</em></td>
<td>Whole animal</td>
<td>20</td>
<td>10% air sat.</td>
<td>3 h</td>
<td>59</td>
<td>van Waversveld <em>et al.</em> (1989a)</td>
</tr>
<tr>
<td>Goldfish</td>
<td><em>C. auratus</em></td>
<td>Whole animal</td>
<td>20</td>
<td>5% air sat.</td>
<td>3 h</td>
<td>53</td>
<td>van Waversveld <em>et al.</em> (1989a)</td>
</tr>
<tr>
<td>Goldfish</td>
<td><em>C. auratus</em></td>
<td>Whole animal</td>
<td>20</td>
<td>Anoxia</td>
<td>3 h</td>
<td>70 to 85</td>
<td>Stangl and Wegener (1996)</td>
</tr>
<tr>
<td>Goldfish</td>
<td><em>C. auratus</em></td>
<td>Whole animal</td>
<td>20</td>
<td>3% air sat.</td>
<td>5 h</td>
<td>55</td>
<td>van Ginneken <em>et al.</em> (2004)</td>
</tr>
<tr>
<td>Crucian carp</td>
<td><em>Carassius carassius</em></td>
<td>Brain slices</td>
<td>12</td>
<td>Anoxia</td>
<td>20 h</td>
<td>37</td>
<td>Johansson <em>et al.</em> (1995)</td>
</tr>
<tr>
<td>Tilapia</td>
<td><em>Oreochromis mossambicus</em></td>
<td>Whole animal</td>
<td>20</td>
<td>5%</td>
<td>8 h</td>
<td>55</td>
<td>van Ginneken <em>et al.</em> (1997)</td>
</tr>
<tr>
<td>Tilapia</td>
<td><em>Oreochromis.</em> mossambicus</td>
<td>Whole animal</td>
<td>20</td>
<td>3% air sat.</td>
<td>1 h</td>
<td>64</td>
<td>van Ginneken <em>et al.</em> (1999)</td>
</tr>
<tr>
<td>European eel</td>
<td><em>Anquilla anguilla</em></td>
<td>Whole animal</td>
<td>20</td>
<td>Anoxia</td>
<td>1 h</td>
<td>70</td>
<td>van Ginneken <em>et al.</em> (2001)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td><em>Oncorhynchus mykiss</em></td>
<td>Hepatocytes</td>
<td>20^a</td>
<td>~4% air sat.</td>
<td>6 to 12 min</td>
<td>46</td>
<td>Rissinanen <em>et al.</em> (2006)</td>
</tr>
<tr>
<td>Zebrafish</td>
<td><em>Brachydanio rerio</em></td>
<td>Whole animal</td>
<td>25</td>
<td>6% air sat.</td>
<td>50 min</td>
<td>Increase by 50^b</td>
<td>Stangl and Wegener (1996)</td>
</tr>
</tbody>
</table>

^a Animals were acclimated to 12°C and metabolic rate was measured in isolated hepatocytes at 20°C.

^b Metabolic rate increased upon exposure to hypoxia.

In studies with more than one level of hypoxia shown, the degree of metabolic rate suppression for the most severe level of hypoxia is shown.
The suppression of protein synthesis has been described in both isolated hepatocytes and fish in vivo in species ranging from the crucian carp (Smith et al., 1996; see Chapter 9), to goldfish (Jibb and Richards, 2008), to the Amazonian oscar (Astronotus ocellatus; Lewis et al., 2007). In the oscar, severe hypoxia exposure (10% air saturation) caused tissue specific decreases in protein synthesis rates that varied from 27% decreases in protein synthesis rate in brain to 60% decreases in heart. In the crucian carp, Smith et al. (1996) also demonstrated substantial decreases in protein synthesis rates in heart, liver, and muscle in response to anoxia exposure and these decreases were, in part, mediated by decreases in RNA transcription rates (Smith et al., 1999). In the goldfish, hypoxia exposure (<0.5% air saturation) caused a very rapid (within 0.5 h) ~70% decline in liver protein translation rate (assessed in cell-free isolates). These decreases in protein synthesis rates in the hypoxic goldfish were mediated through specific phosphorylation of eukaryotic elongation factor-2 (Jibb and Richards, 2008), which halts protein elongation during translation (Figure 10.5).

Few studies have examined how other ATP-consuming processes besides protein synthesis are modified during hypoxia exposure in fish, but some modifications to ion pumping have been noted. In particular, hypoxia-induced decreases in the activity of Na⁺/K⁺-ATPase as observed in some studies (Bogdanova et al., 2005) could represent a substantial ATP saving, but results are conflicting. The crucian carp does not decrease brain Na⁺/K⁺-ATPase activity during anoxia exposure (reviewed in detail in Chapter 9; Hylland et al., 1997) despite increases in the inhibitory neuromodulators GABA (Nilsson, 1992) and adenosine (Nilsson, 1991). This lack of an effect of anoxia/hypoxia exposure on Na⁺/K⁺-ATPase activity in the brain of crucian carp is unlike the response observed in turtles, which suppress the activity of Na⁺/K⁺-ATPase. The differential responses observed in the two champions of anoxia tolerance is probably associated with the fact that crucian carp remains active during anoxia exposure, unlike the comatose turtle (see Chapter 9). Recent work by Richards et al. (2007) demonstrated a substantial decrease in gill Na⁺/K⁺-ATPase activity in the oscar exposed to hypoxia (~5% air saturation) and it was speculated that this decrease was achieved by a post-translational modification to the Na⁺/K⁺-ATPase protein. A similar effect of hypoxia exposure was observed in isolated trout hepatocytes, where hypoxia caused a transient down-regulation of Na⁺/K⁺-ATPase activity (Bogdanova et al., 2005). These authors speculated that decreases in Na⁺/K⁺-ATPase activity in response to hypoxia may be accomplished by local changes in reactive oxygen species, but no precise mechanism was given.
Fig. 10.5. Liver phospho-eEF2 (A), representative phosphoThr56-eEF2 and eEF2 Western blots (B), and protein synthesis rate (C) in goldfish exposed to normoxia and 12 h of hypoxia. [Data from Jibb and Richards (2008) with permission.]
4.3.3. Molecular Responses that Facilitate Metabolic Rate Suppression

As outlined above, decreases in protein synthesis rates are an important response to hypoxia exposure reducing ATP demands and facilitating whole animal metabolic rate suppression. To this end, across a number of tissues, including muscle, liver, and gills, cDNA microarray studies have demonstrated dramatic decreases in mRNA coding for proteins involved in protein synthesis. In muscle, the levels of mRNA coding for elongation factor 2 and several ribosomal proteins have all been shown to be substantially reduced in response to hypoxia exposure (Table 10.1; Gracey et al., 2001). Similarly, in the gills of zebrafish exposed to hypoxia, decreases in mRNA coding for ribosomal proteins have been shown; however, the same study showed a curious accumulation of mRNA coding for elongation factors (van der Meer et al., 2005).

Metabolic energy saving can also be realized through a reduction in movement (Chapter 2) and the maintenance of cellular machinery for movement. Genes involved in muscle contraction including α-tropomyosin, myosin heavy chain, myosin regulatory light chain 2A, skeletal muscle α-actin, and β-actin were, for the most part, all strongly suppressed in response to hypoxia exposure in the mudsucker (Gracey et al., 2001). A similar response was also noted in zebrafish embryos exposed to hypoxia with decreases in mRNA coding for proteins involved in contraction, extracellular matrix, and cytoskeletal proteins (Ton et al., 2003).

Cell growth and proliferation is generally suppressed during hypoxia exposure as a mechanism for ATP conservation. Gracey et al. (2001) observed mRNA increases for a number of genes involved in the suppression of cell growth and proliferation. For example, elevated levels of mRNA for insulin-like growth factor binding protein 1 (IGFBP-1), which regulates the availability of insulin-like growth factors in circulation, were observed in liver. Increases in MAP-kinase phosphates were also observed, including MKP-2, which attenuates the activity of the ERK group of MAP kinases. These kinases are phosphorylated in response to the binding of growth factor to cell-surface receptors and activate a signaling cascade that stimulates cell growth. The importance of inhibition of cell growth as an adaptive response to hypoxia exposure is best illustrated by the elegant work of Sollid and Nilsson (Sollid and Nilsson, 2006; Sollid et al., 2006; see Chapter 9). Briefly, in the crucian carp, hypoxia exposure causes a dramatic increase in gill surface area, mediated primarily by a decrease in cell division and increase in apoptosis in the intralamellar space. However, hypoxia does not yield an increase in mRNA consistent with an increase in cellular apoptosis in zebrafish gills (van der Meer et al., 2005).
Ton et al. (2003) showed repression of several genes involved in cell division such as cyclin G1 and proliferating cell nuclear antigen in zebrafish embryos, which is consistent with observations that hypoxia causes these embryos to undergo developmental arrest and enter a state of suspended animation (Padilla and Roth, 2001).

5. COORDINATING THE METABOLIC AND MOLECULAR RESPONSES TO HYPOXIA

Cell survival during hypoxia exposure requires a metabolic reorganization to decrease ATP demands to match the reduced capacity for ATP production and these metabolic responses must be coordinated temporally otherwise hypoxia exposure will lead to cell death. Several signal transduction cascades have been shown to be activated in response to hypoxia exposure in mammals and other vertebrates (Storey and Storey, 2004), but considerably less work has been done in fishes. In the remaining part of this chapter, I will outline recent advances in the role of one specific signal transduction cascade, the AMP-activated protein kinase, and its role in coordinating the metabolic responses to hypoxia followed by the role of HIF in coordinating the gene expression responses described in this chapter and others.

5.1. AMP-Activated Protein Kinase as a Metabolic Coordinator

Recent evidence has suggested that AMP-activated protein kinase (AMPK) may play a critical role in coordinating the metabolic responses to hypoxia in the hypoxia-tolerant goldfish. AMPK is a heterotrimeric protein kinase comprised of a catalytic subunit (α) and two regulatory subunits, and phosphorylation of AMPK at Thr-172 on the α-subunit activates the protein (Carling, 2004). Activation of AMPK in mammals inhibits energetically expensive anabolic processes including protein synthesis (Horman et al., 2002), glycogen synthesis (Nielsen et al., 2002), and fatty acid synthesis (Hardie and Pan, 2002) rates. Furthermore, activation of AMPK increases skeletal muscle hexokinase activity, GLUT-4 glucose transporter expression (Holmes et al., 1999), and translocation to the membrane (Kurth-Kraczek et al., 1999), and increased phosphofructokinase-2 (PFK-2) activity in rat cardiomyocytes (Marsin et al., 2000), all of which could enhance O₂-independent ATP production. Combined, these actions have led to AMPK being termed the cellular “energy gauge” because of its critical role in maintaining cellular energy balance.
Jibb and Richards (2008) demonstrated that AMPK was activated in the liver of goldfish exposed to severe hypoxia and that there was a close temporal change in \([AMP_{\text{free}}]/[ATP]\) and AMPK activity. Increases in AMPK activity in the liver were associated with an increase in the percent phosphorylation of a well-characterized target of AMPK, eukaryotic elongation factor-2 (eEF2), and decreases in protein synthesis rates measured in liver cell-free extracts (Figure 10.5) suggesting that a disruption of cellular energy status is important for the activation of mechanisms involved in metabolic rate suppression. AMP-activated protein kinase, however, was not activated in muscle, brain, heart, or gill during 12 h of severe hypoxia exposure in goldfish suggesting a tissue-specific regulation of AMPK and metabolic responses to hypoxia (Jibb and Richards, 2008).

5.2. Hypoxia Inducible Factor

Hypoxia-regulated gene expression was described some decades ago, but it wasn’t until 1992 that the \(O_2\)-regulated transcription factor, HIF-1\(\alpha\), was identified as a key regulator of hypoxia-regulated gene expression (Semenza and Wang, 1992). Since its discovery, HIF has been viewed as the molecular master factor of the hypoxic response and a great deal of information is now available on the genes and gene families regulated by HIF (Semenza, 2007; Gardner & Corn, 2008). Many excellent reviews of hypoxia-regulated gene expression and HIF are present in the literature (e.g., Kenneth and Rocha, 2008) including several on fish (e.g., Nikinmaa and Rees, 2005). In the remaining part of this chapter, I will outline HIF regulation in mammals and then describe what is known of HIF function in fish using the work done in mammals as a point of reference.

Hypoxia inducible factor is a heterodimeric transcription factor composed of two subunits; an \(O_2\)-sensitive HIF \(\alpha\) subunit and an \(O_2\) stable HIF \(\beta\) (also referred to as the aryl hydrocarbon receptor nuclear translocator; ARNT). Hypoxia inducible factor \(\alpha\) and \(\beta\) subunits are both members of a very large family of transcription factors known as bHLH/PAS domain-proteins, named because all members of this family contain a basic helix-loop-helix (bHLH) domain as well as one or several PAS domains (domain named after its first members Per, ARNT, and Sim). The bHLH/PAS domain-containing transcription factors constitute a superfamily of transcription factors that are capable of forming homo- and heterodimers through the bHLH and PAS domain and have been implicated in regulating the transcription of genes involved in circadian rhythm, central nervous system development, and induction of hydrocarbon metabolizing enzymes, as well as the cellular responses to hypoxia. Hypoxia inducible factor \(\alpha\),
unlike HIF β, contains an O2-dependent degradation domain (ODD), rendering these proteins labile in the presence of O2.

In mammalian systems, HIF is regulated through the post-translational modifications of HIF α, which affects both protein stability and transactivation (Figure 10.6). The O2-dependent control of HIF α is provided by the actions of two proteins, prolyl hydroxylase (PHDs) and factor inhibiting HIF (FIH; Mahon et al., 2001), both of which are members of the 2-oxoglutarate-dependent dioxygenase superfamily of hydroxylases. These proteins both require iron and 2-oxoglutarate as cofactors or substrates (Schofield et al., 1999), and possess many of the features of an O2-sensitive control mechanism (Land and Hochachka, 1995). Under conditions of normal cellular O2 tensions, HIF α and HIF β are continuously transcribed and translated; however, HIF α is rapidly hydroxylated at two conserved proline residues in the ODD by PHD. HIF α proteins containing hydroxylated

**Fig. 10.6.** Regulation of hypoxia inducible factor by O2. In normoxic cells, propyl hydroxylases (PHD) and factor inhibiting HIF (FIH) enzymes use O2 to hydroxylate key residues on the HIF α subunit in the oxygen-dependent domain (ODD). Hydroxylation of the ODD signals the von Hippel-Lindau (VHL) protein binding leading to ubiquitination and subsequent degradation by the proteosome. The stability of HIF β is not affected by O2 levels. During periods of cellular hypoxia, PDH and FIH are inhibited resulting in the stabilization of HIF α and as HIF α accumulates it dimerizes with HIF β, recruits other co-activators (e.g. p300/CBP) and activates the transcription of genes containing hypoxia response elements in their promoter region.
proline residues are recognized by an E3 ubiquitin ligase, the von Hippel-
Lindau protein (VHL), which promotes Lys48-linked ubiquitination and
targets HIF α for rapid degradation by the cellular proteasome. HIF α is
also hydroxylated at a conserved asparagine residue in the C-terminal-
transactivation domain (cTAD) by FIH, which prevents the recruitment of
the p300/CBP transcriptional coactivators leading to a reduced ability of
HIF α to transactivate and an overall suppression of HIF regulated gene
transcription (Linke et al., 2004). Thus, under normoxic conditions, HIF α
protein is continually made but prevented from accumulating or initiating
transcription through the PHD-mediated ubiquitin-proteasome degradation
and the FIH inhibition of transactivation. In a normoxic cell, HIF α has a
half-life of approximately 5–10 min.

The onset of cellular hypoxia leads to an inactivation of PHD and FIH
and a lack of HIF α hydroxylation. The lack of HIF α proyl hydroxylation
prevents pVHL from recognizing HIF α and initiating the ubiquitin-
regulated protein degradation. Thus, cellular hypoxia leads to an almost
instantaneous stabilization and accumulation of HIF α, which migrates
into the nucleus and dimerizes with HIF β. The HIF α/HIF β dimer then
binds with the p300/CBP coactivator, and the complete complex binds to
specific hypoxia-response elements (HRE) in the promoter regions of target
genes. The absence of asparagine hydroxylation by FIH is permissive for
the HIF dimer to interact with transcriptional coactivators and initiate transcrip-
tion (Lando et al., 2002). Numerous genes have been reported to possess
HRE and associated elements in their 5′ promoter regions and their HIF
regulation has been described; the known hypoxia-induced gene expression
response in fish is described below.

5.2.1. HIF Isoforms

5.2.1.1. HIF α Isoforms. Three HIF α subunit isoforms have been iden-
tified in mammals (designated HIF 1α, HIF 2α, HIF 3α; Gu et al., 1998) and
some differences between these isoforms have been described, although the
precise function of these isoforms has not been fully elucidated. Hypoxia
inducible factor 1α and 2α both contain transactivation domains (cTAD
domains), while HIF 3α appears to lack the cTAD and, as such, it has been
proposed that HIF 3α may act as an inhibitor of HIF 1α and HIF 2α (Bardos
and Ashcroft, 2005). HIF 1α and 2α have been shown to have non-redundant
functions in the cell, and although HIF 1α is the best-studied isoform, recent
studies in mammals have illuminated important roles for HIF 2α in cancer
tumor growth (Carroll and Ashcroft, 2006; Hu et al., 2006). HIF 2α has also
been shown to be expressed at high levels in certain cell types such as vascular
endothelial cells, kidney fibroblasts, hepatocytes, glial cells, interstitial cells
of the pancreas, and epithelial cells of the intestinal lumen (Jain et al., 1998).
The first fish HIF α sequence was determined for the rainbow trout by Soitamo et al. (2001) and since that point, a total of 38 HIF α gene sequences have been identified in fish either through direct sequencing (Powell & Hahn, 2002; Law et al., 2006; Rahman and Thomas, 2007; Rojas et al., 2007; Rytkönen et al., 2007) or as part of genome sequencing projects (see Ensembl genome projects for Zebrafish, Fugu, Tetradon, Medaka, and Stickleback; Figure 10.7). For the most part, fish HIF α protein sequences are slightly shorter than their counterparts in tetrapods. For example, the length of HIF 1α in fish is between 699 and 778 amino acids while in tetrapods HIF 1α is between 800 and 836 amino acids long.

Phylogenetic analysis of available tetrapod, bird, and fish HIF α sequences indicate that the three major classes of HIF α sequences seen in mammals are represented in fish (Figure 10.7). For the most part, within each isoform class, fish sequences group closely together and are distinct from their tetrapod and bird counterparts. The one exception is for HIF 1α from the Russian sturgeon (Acipenser gueldenstaedtii), which groups more closely with the birds (chicken, Gallus gallus) and tetrapods. This grouping of the more pleiomorphic sturgeon HIF 1α with tetrapods and birds suggests that the more derived teleost fish species may have a faster changing HIF 1α sequence.

Several HIF 4α isoforms have been identified in fish (Law et al., 2006; also see orange-spotted grouper; Epinephelus coioides), however their identification at the time was based upon a lack of similarity to the scant fish and tetrapod HIF sequences. Since that time, the proliferation of available HIF α sequences and the phylogenetic analysis performed in Figure 10.7 suggests that previously identified HIF 4α sequences are in fact HIF 3α sequences.

Among the isoforms identified in fish, all the appropriate functional domains can be identified. For example, sequence analysis of deduced amino acid sequence of fish HIF 1α genes reveals the presence of four major functional domains including the bHLH domain, two PAS domains (PAS-A and PAS-B), ODD domain, and the DNA-binding domain termed cTAD. These four major functional domains are the same as those seen in tetrapod and bird HIF 1α sequences. Sliding window analysis of 11 fish HIF 1α gene sequences clearly demonstrates that the amount of amino acid sequence variability between fish species is lowest at the four major functional domains (Figure 10.8). These analyses suggest that the amino acid sequence of the important functional domains is well conserved across fish species. Sites showing a high degree of sequence variability occur in areas that have not been identified as important for HIF 1α function. Given the high degree of similarity between fish, tetrapod, and bird sequences it seems reasonable to generalize that HIF 1α functions in fish in much the same way as it does in tetrapods (Figure 10.6).
Fig. 10.7. Phylogenetic analysis of HIF α isoform amino acid sequences from fish, tetrapods, and birds. The phylogeny was created from deduced amino acid sequences from GenBank or Ensembl: Oncorhynchus mykiss HIF 1 (AF304864); Thymallus thymallus HIF 1 (ABO26714);
5.2.1.2. HIF β Isoforms. At least two HIF β isoforms have been identified in tetrapods; HIF 1β, which is ubiquitously expressed in most tissues, and HIF 2β, which is primarily restricted to nervous system and kidneys at specific developmental stages (Hirose et al., 1996). In fishes, a total of 14 HIF β isoforms have been identified and in general they group closely with other known HIF β isoforms identified in tetrapods and birds (Figure 10.9). At the sequence level, HIF β is similar to HIF α in that it is a member of the bHLH/PAS group of transcription factors and both isoforms possess bHLH and PAS domains. HIF β also possesses a terminal activation domain (AD). Sequence analysis of all available fish HIF β sequences reveals a high degree of sequence conservation in these well-identified functional domains (Figure 10.10). Overall, this high degree of sequence similarity and the conservation of important regulatory and functional domains suggest that fish HIF β probably functions in a similar fashion to its tetrapod and bird orthologs.

Esox lucius HIF 1 (ABO26715); Micropogonias undulates HIF 1 (ABD32158); Perca fluviatilis HIF 1 (ABO26717); Stizostedion lucioperca HIF 1 (ABO26718); Gymnocephalus cernuus HIF 1 (ABO26716); Pachycephala brachycephalum HIF 1 (AAZ52828); Zoarcus viviparus HIF 1 (AAZ52832); Dicentrarchus labrax HIF 1 (AAZ95453); Epinephelus coioides HIF 1 (AAW29027); Gasterosteus aculeatus HIF 1 (ABO26719); Orzyas latipes HIF 1 (ENSORLT0000004404); Rattus norvegicus HIF 1 (NP.075578); Tetraodon nigroviridis HIF 1 (ENSTNIG00000017339); Takifugu rubripes HIF 1 (ENSTRU00000012093); Orzyas melastigma HIF 1 (ABC47310); Ctenopharyngodon idella HIF 1 (AAR95697); Platichthys flesus HIF 1 (ABO26720); Ictalurus punctatus HIF 1 (AAZ75952); Danio rerio HIF 1 (AA91619); Cyprinus carpio HIF 1 (ABV59209); Carassius carassius HIF 1 (ABC24677); Gymnocypris przewalskii HIF 1 (AAW69834); Aspius aspius HIF 1 (ABO26713); Acipenser guledenstaedtii HIF 1 (ABO26712); Rana temporaria HIF 1 (ABY86629); Mustelus canis HIF 1 (ABY86628); Gallus gallus HIF 1 (NP.098628); Xenopus laevis HIF 1 (ABF71072); Rattus norvegicus HIF 1 (NP.077335); Eospalax baileyi HIF 1 (ABB17537); Eospalax cannus HIF 1 (ABQ53550); Microtus oeconomus HIF 1 (AAY27087); Spalax juraei HIF 1 (CAG29396); Spermophilus tridecemlineatus HIF 1 (AAU14021); Mus musculus HIF 1 (BAA20130); Oryctolagus cuniculus HIF 1 (NP.010176251); Pantholops hodgsonii HIF 1 (AAX89137); Bos grunniens HIF 1 (ABH06559); Mus musculus HIF 1 (AAH26139); Bos taurus HIF 1 (NP.776764); Homo sapiens HIF 1 (AAF20149); Macaca fascicularis HIF 1 (BAE01417); Fundulus heteroclitus HIF 2 (AAL95711); Micropogonias undulates HIF 2 (ABD32159); Takifugu rubripes HIF 2 (ENSTRUT00000013648); Ctenopharyngodon idella HIF 2 (AAT76668); Bos taurus HIF 2 (BAA78676); Mus musculus HIF 2 (NP.034267); Trematomus hansoni HIF 2 (AAZ52830); Ictalurus punctatus HIF 2 (ABK27926); Chionodraco myers HIF 2 (AAZ52827); Coturnix coturnix HIF 2 (AAF20152); Ctenopharyngodon idella HIF 4 (AAR95698); Danio rerio HIF 3 (AAQ94179); Ictalurus punctatus HIF 3 (AAZ75953); Epinephelus coioides HIF 4 (AAW29028); Rattus norvegicus HIF 3 (NP.071973); Mus musculus HIF 3 (NP.058564); Bos taurus HIF 3 (NP.001098812); Homo sapiens HIF 3 (NP.690008); Takifugu rubripes HIF 3 (ENSTRUT00000012549); Tetraodon nigroviridis HIF 3 (ENSTNIT0000009762); Orzyas latipes HIF 3 (ENSORLT0000002500). Sequences were aligned using ClustalW and phylogenetic analysis was performed using the neighbor-joining methods with complete deletion of gaps using MEGA2 software (Kumar et al., 2001). The support for each node was assessed using 500 bootstrap replicates and are presented at each branch point. Bold-face type indicates fish sequences.
In addition to its role in hypoxic signaling, HIF 1β, or rather ARNT, is known to play an important role in regulating gene expression changes in response to toxic aryl-hydrocarbon exposure (Hahn et al., 2006). A number of the gene expression responses to aryl-hydrocarbon exposure are similar to those observed in response to hypoxia exposure including increases in lactate dehydrogenase gene expression. HIF 1β regulates aryl hydrocarbon-mediated changes in gene expression through the binding of the aryl hydrocarbon to a specific receptor, the aryl hydrocarbon receptor (AHR). The AHR then binds to its partner, HIF 1β, and the AHR/HIF 1β heterodimer moves to the nucleus where it binds to xenobiotic responsive elements (XREs). Binding of the AHR/HIF 1β heterodimer to XRE regions adjacent to aryl hydrocarbon-inducible genes increases their transcription. Because HIF 1β is known to be involved in the responses to hypoxia and aryl-hydrocarbon exposure, possible interactions between responses may exist. Kraemer and Schulte (2004) demonstrated an antagonistic interaction
Fig. 10.9. Phylogenetic analysis of HIF $\beta$ isoform amino acid sequences from fish, tetrapods, and birds. The phylogeny was created from deduced amino acid sequences from GenBank or Ensembl: *Pongo abelii* HIF 1$\beta$ (NP_001125275); *Gasterosteus aculeatus* HIF 1$\beta$
between exposure to PCBs (3,3',4,4'-tetrachlorobiphenyl) and hypoxia (~15% air saturation) in killifish and suggested that prior PCB exposure could make these fish less tolerant of environmental hypoxia.

5.2.1.3. PHD Isoforms. Four PDH isoforms have been identified in mammals, numbered PDH 1 to 4, and so far only PDH 1, 2, and 3 have been shown to hydroxylate HIF. Biochemical analysis has shown PHD 2 to have a higher affinity for HIF 1α, whereas PDH 1 and PDH 3 have higher affinity for HIF 2α (Appelhoff et al., 2004). Prolyl hydroxylase sequences have been found in fish as a result of genome sequencing projects, but to date no study has explicitly characterized the sequence or function of PHD isoforms in fish. This will undoubtedly be an important and fruitful area of research in the next few years as PHDs are now considered the cellular O₂ sensors responsible for initiating the HIF response.

5.2.2. Regulation of HIF Activity in Fish

The O₂-dependent regulation of HIF in fish has received remarkably little attention since the literature was reviewed by Nikinmaa and Rees (2005). However, given the available data on sequence similarity between tetrapod and fish HIF α and β sequences it seems reasonable to speculate that the same or similar mechanisms of O₂-dependent regulation of HIF shown in Figure 10.6 are at play in fish. Specifically, as pointed out by Rahman and Thomas (2007) for Atlantic croaker (Micropogonias undulates) and shown in

-Sequences were aligned using ClustalW and phylogenetic analysis was performed using the neighbor-joining methods with complete deletion of gaps using MEGA2 software (Kumar et al., 2001). The support for each node was assessed using 500 bootstrap replicates and is presented at each branch point. Bold-face type indicates fish sequences.
Figure 10.8, there is a high degree of sequence similarity in the core O$_2$-dependent degradation domain regions of fish HIF $\alpha$ sequences, suggesting a similar mechanism of HIF degradation to that in other vertebrate species.

The first and only study to address the issue of O$_2$-dependent regulation of HIF in fish was that of Soitamo et al. (2001), which demonstrated that although HIF 1$\alpha$ was present under normoxic conditions (air saturation) in rainbow trout and salmon cell lines, the levels of HIF 1$\alpha$ protein increased during hypoxia exposure. Oddly, however, the maximum levels of HIF 1$\alpha$ protein were noted in cells cultured at 5% O$_2$, which as the authors pointed out is similar to typical venous PO$_2$. These data suggest that in vivo, HIF 1$\alpha$ may accumulate under what should be considered as normoxic conditions in tissues. Additional research is needed to understand how HIF functions in fish cells and whether there are differences in O$_2$ sensitivity in HIF 1$\alpha$-regulated gene expression among fish that vary in hypoxia tolerance.

5.2.3. Hypoxia-regulated HIF $\alpha$ mRNA Expression

Unlike in mammals, where there appears to be little or no regulation of HIF at the mRNA level, hypoxia-induced changes in HIF $\alpha$ mRNA and protein expression have been noted in several fish species. Law et al. (2006) examined the mRNA and protein levels of two HIF $\alpha$ isoforms (1 and 3; note that these authors incorrectly named HIF 3$\alpha$ as HIF 4$\alpha$) from the hypoxia-tolerant grass carp (Ctenopharyngodon idella) and showed substantial increases in HIF 1$\alpha$ mRNA in gill and kidney after 4 h exposure to $\sim$7% air saturation compared with normoxia-exposed fish. In the same fish, no or
few changes in HIF 1α were noted in brain, eye, gill, heart, kidney, liver, and muscle. On the other hand, substantial increases in the HIF 3α isoform (identified as HIF 4α) were observed during hypoxia exposure in all tissues examined. Similarly, Rahman and Thomas (2007) demonstrated that both HIF 1α and HIF 2α from the hypoxia-tolerant Atlantic croaker were hypoxia responsive in ovaries during short-term (3–7 days at ~20% air saturation) and longer-term hypoxia exposure (3 weeks at ~20–40% air saturation). There does not, however, appear to be a good relationship between hypoxia tolerance and HIF α expression, although the available data are limited. Specifically, HIF 1α mRNA levels have also been shown to increase in the liver of the hypoxia-sensitive sea bass (Dicentrarchus labrax; Terova et al., 2008) during both acute hypoxia exposure (4 h at ~20% air saturation) and 15 days of chronic hypoxia (~50% air saturation).

5.2.4. Relationship Between HIF Function and Hypoxia Tolerance in Fish

The fact that there is enormous variation in hypoxia tolerance among fish species raises the question of whether there is a relationship between HIF function and hypoxia tolerance. In fact, careful comparisons among fish species known to vary in hypoxia tolerance open the possibility of elucidating which aspects of HIF function are adaptive and thus potentially most important in dictating hypoxia tolerance. Surprisingly however, most of our current understanding of HIF regulation and function comes from mammalian models, which typically only experience hypoxia as a result of disease such as cancer (Gort et al., 2008).

To begin to address the question of whether HIF structure or responsiveness to hypoxia differ among hypoxia-sensitive and hypoxia-tolerant fish species, Rytkönen et al. (2007) sequenced HIF 1α from nine species of fish that varied in lifestyle related to O₂ requirements (hypoxia tolerance was not quantified). Analysis of sequence variation among the available fish HIF 1α amino acid sequences showed that there was no clear protein signature associated with O₂ requirements (Rytkönen et al., 2007). Further analysis of these sequences and others revealed that the overall evolutionary rate in teleost HIF 1α was approximately twice as fast as the predicted evolutionary rate in mammalian HIF 1α (Rytkönen et al., 2008). Despite the faster sequence divergence, however, crucial functional domains in HIF 1α (Figure 10.6) were found to be under stringent purifying selection in all vertebrates. As a result, the faster sequence divergence occurred in the less crucial areas of sequence. Some evidence for positive selection on HIF 1α amino acid sequence was observed, but was not associated with sequence variation in the O₂ sensitive ODD, but was associated with the bHLH/PAS domains.
5.2.4. Oxygen-dependent Gene Expression

Hypoxia exposure in fish is well known to initiate a complex suite of gene and protein expression responses, many of which have been outlined above (see Table 10.1; Gracey et al., 2001; Ton et al., 2002, 2003; Bosworth et al., 2005; van der Meer et al., 2005). However, in many cases a direct link between changes in gene or protein expression and the transcriptional regulator HIF has not been directly assessed, therefore the reader is cautioned against assuming all responses described above are mediated by HIF. In reality, remarkably few studies, especially in fish, have focused on identifying functional HRE in the promoter regions of the hypoxia-responsive genes. The only definitive studies conducted in fish that have shown a direct relationship between HIF and hypoxia-regulated gene expression is for insulin-like growth factor binding protein in zebrafish (Kajimura et al., 2005, 2006). In mammalian and carcinoma cell lines, however, HIF has been directly implicated in regulating the expression of genes involved in a number of physiological and biochemical responses to hypoxia (outlined above).

Patterns of gene expression in response to hypoxia exposure can vary between tissues and in some cases the differences can be dramatic. Ju et al. (2007) using an 8046 gene microarray showed substantial tissue-specific gene regulation and few consistent responses between tissues. In response to hypoxia exposure, 501 genes in the brain, 442 genes in the gills, and 715 genes in the liver were differentially expressed in hypoxia-exposed medaka (Oryzias latipes) and there were a number of pathways affected (Table 10.1). Among the up-regulated genes there were remarkably few overlapping genes with 24, 21, and 20 genes showing the same expression patterns between brain and gill, brain and liver, and gill and liver, respectively (Figure 10.11). Of the genes that were shown to be down-regulated, 65, 24, and 26 genes were common between brain and gill, brain and liver and gill and liver, respectively (Ju et al., 2007). Only nine genes in total changed in a consistent fashion across all tissues examined. Of all the tissues examined, liver showed the greatest number of differentially expressed genes.

6. CONCLUSIONS AND PERSPECTIVES

Hypoxia survival requires a rapid reorganization of physiological and biochemical systems to either maximize O2 uptake from the hypoxic environment to support the maintenance of a routine metabolic rate or cellular adjustments to function under O2-limiting conditions. Survival under O2-limiting conditions requires a cellular metabolic reorganization to reduce ATP consumption through a regulated metabolic rate suppression to
match the limited capacity for O₂-independent ATP production. As outlined above, controlled metabolic rate suppression is essential to extend the length of time that can be supported by the limited levels of fermentable fuels. Thus, it appears reasonable to speculate that the degree of metabolic rate suppression and the quantity of stored fermentable fuel is likely strongly selected for in hypoxia-tolerant fishes. Indeed, this chapter has outlined and summarized the available information on the degree of metabolic rate suppression in a variety of fish species as well the quantity of tissue glycogen and, broadly speaking, there was a reasonable relationship between fish lifestyle (that being sluggish, hypoxia-tolerant carp species c.f. athletic, intolerant salmonid species, for example) and stored fermentable fuels, but the relationship between metabolic rate suppression and hypoxia tolerance is, however, oddly not clear. This is primarily because of the scant data available on the topic. Further still, the study of HIF in fish and hypoxia-regulated gene expression has been fruitful in demonstrating that HIF function in fish appears at least superficially similar to that observed in mammals, but the relationship between HIF function and hypoxia tolerance is still lacking. Despite the wealth of information available on the metabolic and molecular responses of a variety of fish species to hypoxia, we are still far from a unified concept of the important adaptations underlying hypoxia tolerance. However, fish provide an incredibly tractable system to understand the evolution of hypoxia tolerance because of the incredible diversity of fishes as well as their diverse O₂ habitats.

Fig. 10.11. Venn diagram showing differentially expressed genes in medake during hypoxia exposure. (A) Number of up-regulated genes in response to hypoxia exposure; (B) number of down-regulated genes in response to hypoxia exposure. [Data from Ju et al. (2007) with permission.]
REFERENCES


10. METABOLIC AND MOLECULAR RESPONSES TO HYPOXIA


