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Review

Some insights into energy metabolism for osmoregulation in fish ☆

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ABSTRACT

A sufficient and timely energy supply is a prerequisite for the operation of iono- and osmoregulatory mechanisms in fish. Measurements of whole-fish or isolated-gill (or other organs) oxygen consumption have demonstrated regulation of the energy supply during acclimation to different osmotic environments, and such regulation is dependent on species, the situation of acclimation or acclimatization, and life habits. Carbohydrate metabolism appears to play a major role in the energy supply for iono- and osmoregulation, and the liver is the major source supplying carbohydrate metabolites to osmoregulatory organs. Compared with carbohydrates, the roles of lipids and proteins remain largely unclear. Energy metabolite translocation was recently found to occur between fish gill ionocytes and neighboring glycogen-rich (GR) cells, indicating the physiological significance of a local energy supply for gill ion regulatory mechanisms. Spatial and temporal relationships between the liver and other osmoregulatory and non-osmoregulatory organs in partitioning the energy supply for ion regulatory mechanisms during salinity challenges were also proposed. A novel glucose transporter was found to specifically be expressed and function in gill ionocytes, providing the first cue for investigating energy translocation among gill cells. Advanced molecular physiological approaches can be used to examine energy metabolism relevant to a particular cell type (e.g., gill ionocytes), and functional genomics may also provide another powerful approach to explore new metabolic pathways related to fish ion regulation.

Contents

1. Introduction

Energy supply is a limiting factor for physiology processes. Therefore, studying energy metabolism for a particular physiological process is an important issue to relevant physiological research. Teleosts, inhabiting environments with various salinities, have

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complicated and sophisticated mechanisms of osmoregulation to maintain the internal osmotic and ionic homeostasis, which allows normal functioning of cellular and physiological processes and survival (Evans et al., 2005; Hwang and Lee, 2007). These iono- and osmoregulatory processes are achieved by various enzymes and transporters, and the synthesis and operation of these transport-related proteins are highly energy consuming. In general, timely additional energy is required particularly in a situation of a fluctuating environment to which an organism responds by synthesizing and activating related enzymes, transporters, and/or enzymes. Energy metabolism for osmoregulation is a research topic with a long history not only for its scientific importance to fish physiology but also because of its relevance to aquaculture. In the present review, we do not examine details of the extensive literature. Instead, our efforts focus on discussing the nutrient metabolisms and the metabolite transport, particularly in carbohydrates in which recent progress has been made or which require further study.

2. Oxygen consumption

The term "metabolism", in its broadest sense, is the sum total of all chemical reactions occurring in an organism, and measuring the oxygen consumed in the entire organism is a commonly used method to indirectly monitor its metabolic rate (Randall et al., 2002). Using whole-fish oxygen consumption to examine the effects of different environmental salinities has a long history, and the energy required to acclimate to a salinity condition can be derived from changes in oxygen consumption.

Many previous studies indicated that acclimating to different salinities causes changes in oxygen consumption, but it is difficult to reconcile differences in those results obtained likely due to differences in species, duration of acclimation, experimental design, and details of the measurement methodology (see Febry and Lutz, 1987; Moser and Hettler, 1989; Morgan and Iwama, 1991; Aristizabal-Abud, 1992; Ron et al., 1995; Woo and Kelly, 1995; Haney and Nordlie, 1997; Morgan et al., 1997; Morgan and Iwama, 1998; Swanson, 1998; Plaut, 2000; Sardella et al., 2004; Gracia-Lopez et al., 2006; Wagner et al., 2006; Wood et al., 2007). Indeed, Morgan and Iwama (1991) summarized five energy metabolism patterns from previous studies: (1) there is no change in the metabolic rate; (2) the metabolic rate is minimum in isotonic salinity but increases in different salinities; (3) a linear relation exists between the metabolic rate and fluctuant salinity; (4) the metabolic rate increases in fresh water (FW) and is reduced in a condition of isotonic salinity; and (5) the highest metabolic rate occurs in seawater (SW). However, Morgan and Iwama (1991) concluded that: (1) in salmonids, the higher metabolic rates in higher water salinities reflect significant energetic costs, accompanied by declining growth rates, and these correlate very well with changes in oxygen consumption, and (2) life habits appear, to a certain extent, to determine the type of metabolic response to salinity changes, i.e., the lowest metabolic rates are associated with the environment in which a species is most commonly found. As summarized by Boef and Payan (2001), 20%–68% of the total energy expenditure is estimated to be consumed by osmoregulation in different species. However, Morgan and Iwama (1991) stated that estimates of osmoregulation costs based on whole-fish oxygen consumption should consider the effects by other metabolic processes which respond to changes in salinity. During SW acclimation, several hormones are known to affect different pathways of energetic metabolism (Sangiao-Alvarellos et al., 2006a,b, 2007), and other non-osmoregulatory organs (like the brain) also show changes in energetic metabolism (Sangiao-Alvarellos et al., 2005; Polakof et al., 2006).

Whole-fish oxygen consumption cannot distinguish the partitioning of energy costs to a particular organ responsible for fish iono-/osmoregulatory mechanisms. Some studies using isolated/perfused gill-arch preparations measured the metabolic rates specific for gills in FW- and SW-acclimated Atlantic cod (*Gadus morhua*) (Johansen and Pettersson., 1981), Atlantic salmon (*Salmo salar*) (McCormick et al., 1989), European flounder (*Platichthys flesus*) (Lyndon, 1994), tilapia (*Oreochromis mossambicus*) (Perry and Walsh, 1989), and cutthroat trout (*Oncorhynchus clarki clarki*) (Morgan and Iwama, 1999). According to Perry and Walsh (1989), CO_2 production (another indicator for oxidative metabolism), Na^+–K^+-ATPase activity and ionocyte number were all higher in the isolated-gill cells of SW tilapia than in that of FW ones. Assuming the majority of the H^+-ATPase and Na^+–K^+-ATPase to be related to active NaCl uptake, ouabain-sensitive and bafilomycin-sensitive oxygen consumption levels in isolated trout (*O. clarki clarki*) gills (Morgan and Iwama, 1999) were found to be similar to theoretical values determined by Eddy (1982) and Kirschner (1995), who used a thermodynamic or molecular approach based on ATP/O_2 consumption by ionic fluxes. Morgan and Iwama (1999) concluded that the contribution of total-gill-arch oxygen consumption to the whole-animal oxygen uptake appears to be relatively small (<4%). From the data of McCormick et al. (1989), oxygen consumption of the gills constitutes <20% of that of a whole fish of Atlantic salmon (*S. salar*). On the other hand, Boef and Payan (2001) discussed in detail the methodologies of those studies using isolated/perfused gill-arch preparations, and addressed their constraints of being unable to accurately estimate the energy requirement for all transport processes. So far, data on oxygen consumption have provided important information about the energy requirements in a whole fish or an isolated organ during acclimation to different salinities, although the methodologies used have their limitations.

3. Energetic metabolism

Previous studies on oxygen consumption during salinity acclimation clearly indicated the need for additional energy for modulating and stimulating ion transport mechanisms in fish. Metabolic reorganization and alterations in intermediary metabolic pathways occur in an effort to meet the increased energy demands associated with acclimation to new environmental salinities. The oxygen:nitrogen (O:N) atomic ratio obtained from oxygen consumption and ammonia excretion is an indicator of metabolic processes using different energy substrates. Changes in the O:N ratio reflect that fish use different metabolic substrates as energy sources for metabolic processes in various environments (Mayzaud and Conover, 1988; Gracia-Lopez et al., 2006; Rocha et al., 2007). Values of 3–16 of the ratio indicate predominately protein substrate oxidation, values of 16–60 reveal a mixture of proteins and lipids as substrates, while values of >60 represent a predominant lipid substrate for meeting elevated energy demands (Mayzaud and Conover, 1988). The O:N ratio has been reported to change depending on environmental salinities (Rocha et al., 2005; Gracia-Lopez et al., 2006; Rocha et al., 2007). In common snook (*Centropomus undecimalis*) acclimated to 35 parts per thousand (ppt) SW, a mixture of protein, lipids, and carbohydrates was suggested to be the metabolic substrates used by the fish, while a greater preference for proteins was estimated at lower salinities (Gracia-Lopez et al., 2006). On the other hand, Rocha et al. (2005, 2007) indicated that juveniles of the fat snook (*Centropomus parallelus*) rely on lipids based on an elevated O:N ratio, and concluded the importance of lipids for meeting the metabolic requirements after long-term acclimation to a salinity of 30 ppt. Although previous results differed depending on species, salinities, and acclimation duration, data on the O:N ratio have demonstrated the utilization of different metabolic substrates as energy supplies during SW acclimation.

3.1. Lipids

Lipids not only play an essential role as membrane components, but also are important and rich energy sources. In fish, the major components of lipids are polyunsaturated fatty acids (PUFAs), particularly the *n*-3 PUFA, docosahexanoic acid (Mourente and Tocher,

1992, 1998; Tocher, 1993). PUFAs and triacylglycerols (TGs) have been documented to be the main forms of lipid storage in fish (Ho et al., 2003). However, other types of TGs, such as glyceryl ether analogues and alkoxydiacylglycerol, have also been documented in some fish species (Sheridan, 1994; Ho et al., 2003). The major sites for lipid storage in fish include mesenteric fat cells as in mammals, and also the liver and red muscle. In some marine species, lipid deposits also occur in the skeleton and affect hydrodynamic lifting (Pelster, 1998). Various lipases, including TG lipase, lipoprotein lipase, and lysosomal lipase, are responsible for lipolysis in fish (Sheridan, 1988, 1994), and the process of lipolysis and the transport of the resulting metabolites in fish are generally similar to those in mammals (Ho et al., 2003). Maintenance of energy homeostasis during food deprivation and exhaustion in fish is directly related to the capacity of the liver to mobilize lipid reserves, at least during the initial stages of fasting, which also depends on subsequent hepatic metabolic activation (Sheridan and Mommsen, 1991). Compared with mammals, fish metabolize fatty acids more readily and efficiently, and therefore, fish can exist for a long period of time under conditions of food deprivation (Skonberg et al., 1994; Figueiredo-Garutti et al., 2002).

Upon encountering osmotic stress, successful activation of the compensatory acclimation mechanisms in euryhaline fish depends on a substantial energetic reorganization over a relatively short period of time. Some works have discussed lipid metabolism in terms of fish osmoregulation. Measurements have been conducted to analyze the effects of hyperosmotic stress on the activities and/or contents of several parameters: non-esterified fatty acids (NEFAs) in the plasma, carnitine palmitoyl transferase (CPT), 3-hydroxyacyl CoA dehydrogenase (HOAD), and malic enzyme (ME) in the gills, liver, and red muscle of Arctic char (*Salvelinus alpinus*); TG in the plasma of gilthead sea bream (*Sparus auratus*) and Atlantic salmon (*S. salar*) (Nordgarden et al., 2002; Sangiao-Alvarellos et al., 2003b, 2005; Bystriansky et al., 2007). However, some contradictory phenomena have been reported. Levels of NEFAs and TGs in the plasma did not change during 96-h acclimation to SW, nor did the activities of CPT, HOAD, or ME in any of the tissues examined in either sea bream (*S. auratus*) or Arctic char (*S. alpinus*), suggesting that lipid metabolism was unchanged (Nordgarden et al., 2002; Sangiao-Alvarellos et al., 2005; Bystriansky et al., 2007). These results are contrary to the findings of Sangiao-Alvarellos et al. (2003a) and Aas-Hansen et al. (2005) who reported an increase in the plasma TG contents of sea bream (*S. auratus*) and Arctic char (*S. alpinus*) after long-term SW acclimation, and an increase in the liver HOAD activity during the downstream migration (prior to moving into seawater) of Arctic char (*S. alpinus*). Increases in the TG content and HOAD activity suggest an enhanced capacity for oxidizing lipids in those species. On the other hand, in the liver and muscle of Atlantic salmon (*S. salar*), TG levels are known to decrease during SW acclimation (Sheridan, 1988). These inconsistencies in previous studies on lipid metabolism during salinity challenges may have partially been due to differences in species, acclimation salinities, and exposure durations, as well as other experimental conditions. This also reflects the great mystery which exists about the lipid utilization for oxidative fuels and membrane synthesis in fish to carry out iono- and osmoregulation upon exposure to environmental osmotic variations.

3.2. Proteins

Philips (1969) suggested that 70% of dietary calories of feed in trout (*O. mykiss*) are from protein; thus a greater percentage of dietary protein is metabolized for energy demands than is utilized for body protein synthesis. Dietary protein is generally absorbed in an intact macromolecular form or as small peptides in the piscine gastrointestinal tract (McLean and Ash, 1987; McLean et al., 1999). The major proportion of absorbed intact proteins and peptides are believed to be hydrolyzed into oligopeptides at the gut brush border (Sire and Vernier, 1992) through the actions of proteases and peptidases, including trypsins, and chymotrypsins (Gudmundsdóttir and Pálsdóttir, 2005). The oligopeptides are then converted to small peptides or free amino acids, which are absorbed into the bloodstream. Taking trypsins as an example, this serine protease has been demonstrated to be beneficial for fish acclimatizing to cold environments. Fish trypsins show higher catalytic efficiencies than do their mammalian analogues (Schrøder Leiros et al., 2000; Roach, 2002; Gudmundsdóttir and Pálsdóttir, 2005). Hence, the metabolism of proteins and the resulting metabolites provide energy sources that are important for fish acclimating to severe environments (Aragão et al., 2004; Cara et al., 2007).

Mobilization of protein metabolites within the whole body also appears to be important for osmoregulation. Amino acids, such as taurine and glycine, can be mobilized to serve as osmoregulatory intracellular solutes called "compatible solutes" in marine fish (Fugelli et al., 1995; Bystriansky et al., 2007; Fiess et al., 2007). In mammalian renal cells, taurine, was found in most osmotically stressed cells as an osmolyte (Somero, 1986). Some studies have examined the mobilization of taurine in teleosts. Taurine efflux was measured in flounder (*P. flesus* L.) erythrocytes (Fugelli and Thoroed, 1986) and goldfish (*Carassius auratus*) renal tissue (Fugelli et al., 1995). Taurine, as an osmolyte, may play some role in intracellular homeostasis in fish during salinity challenges, but the overall mechanism and its regulation remain unclear.

Amino acids are not only oxidized for ATP production but are also used to synthesize macromolecules (e.g., proteins and membranes) in gills and other osmoregulatory organs during SW acclimation. However, the role of amino acids as oxidative substrates has been largely neglected in most previous studies that examined the effects of environmental salinity on the amino acid contents of fish plasma or tissues. Only a few reports have discussed integrated changes in amino acid catabolism in osmoregulatory organs during SW acclimation (Mommsen, 1984; Kultz and Jurss, 1993; Bystriansky et al., 2007). In early investigations, the transdeamination of non-essential amino acids (NEAAs), like aspartate and alanine, was known to be important pathways for energy production in fish (Walton and Cowey, 1982). Recent research on climbing perch (*Anabas testudineus*) showed remarkable accumulations of both aspartate and alanine in muscles after 6 d of acclimation to 30 ppt SW (Chang et al., 2007a). Bystriansky et al. (2007) studied gills and found that aspartate aminotransferase activity increased following salinity change, suggesting an enhanced ability to utilize aspartate. In addition, Bystriansky et al. (2007) also showed increases in alanine concentrations in gills and white muscles, and a decrease in the plasma after acclimation to SW for 96 h. Those results imply that the osmoregulatory tissue (gills) as well as the non-osmoregulatory tissue (white muscle) may accumulate alanine; however whether alanine is used as an energy source in those tissues remains to be clarified. Mommsen et al. (1980) proposed that alanine may be the preferred carrier of amino acid nitrogen for inter-tissue transport, as several amino acids can be converted to alanine, released to the blood, and used as a fuel source in other tissues. The subsequent oxidation of aspartate and alanine by their respective aminotransferases can lead to glutamate accumulation in conditions without glutamate dehydrogenase (GDH) deamination (Mommsen, 1984). Therefore, glutamate may also provide another energy source for osmoregulation. GDH activity did not increase in the gills of Arctic char acclimated to SW for 96 h (Bystriansky et al., 2007), supporting the notion proposed by Mommsen (1984). On the other hand in tilapia (*O. mossambicus*), both GDH activity and glutamate content were reported to increase in isolated-gill epithelial cells following long-term SW acclimation (Kultz and Jurss, 1993). One cannot exclude the possibility that increased gill glutamate levels may also originate from the plasma. Walton and Cowey (1977) showed that gills of rainbow trout (*O. mykiss*) are capable of taking up glutamate from the circulation. Oxidation of branched-chain amino acids (BCAAs) by BCAA transaminase also produces glutamate, resulting in further accumulation of glutamate in cells. Levels of three BCAAs, including leucine, isoleucine, and valine, were found to significantly increase in fish plasma following 5-d

acclimation to SW (Bystriansky et al., 2007; Chang et al., 2007a). Taking all of these results into consideration, glutamate may be used as an energy substrate for osmoregulation in fish gills during SW acclimation; however, whether it originates from gill cells themselves or other tissues may depend on the species.

As discussed above, most previous studies addressed the roles and regulation of some NEAAs in osmoregulatory mechanisms in fish gills during SW acclimation. Indeed, data on the NEAA proportion in the plasma also support those findings. The NEAA proportion in plasma was reported to decrease in rainbow trout (*O. mykiss*) after a long-term acclimation to SW (Kaushik and Luquet, 1979; Ballantyne, 2001). After re-calculating the concentrations of each NEAA in studies by Bystriansky et al. (2007) and Chang et al. (2007a), the NEAA proportion was found to decline in the plasma, but increase in the gills, white muscles, red muscles, red blood cells, and/or liver of Arctic char and climbing perch after SW acclimation. The role of NEAAs in fish osmoregulation appears to be more dominant than that of essential amino acids (EAAs), but further studies are required to confirm this.

3.3. Carbohydrates

Carbohydrates from food are generally digested and absorbed by the intestines, and the resultant monosaccharide metabolites in the bloodstream are transported to other tissues via specific monosaccharide transporters. Monosaccharide metabolites (mainly glucose) directly supply cells' energy demands, and some portion of them are stored as glycogen, a polysaccharide, by the process of glycogenesis in the liver and other organs (Chesley et al., 1998; Choi et al., 2003; Milligan, 2003). Glycogen in these organs is degraded into monosaccharides again when additional energy is required. This is generally true for vertebrates including fish.

The carbohydrates used in energy metabolism related to fish osmoregulation have been studied more extensively than lipids and proteins. There is a long history discussing relationships between carbohydrate metabolism and ion regulation in fish. Soengas and colleagues thoroughly investigated the gilthead sea bream (*S. auratus*), a euryhaline teleost capable of living in environments with different salinities, by examining changes in energy metabolism during salinity challenges. When acclimated to 38 ppt–55 ppt SW for 14 d, levels of glucose and lactate in the plasma and the activities of gill hexokinase and liver glycogen phosphorylase (GP) increased, indicating the enhanced mobilization of glycosyl units from glycogen stores, which results in a higher capacity to export glucose from the liver and increased use of exogenous glucose in gills and other high-energy-consuming organs (Sangiao-Alvarellos et al., 2003b, 2005). Subsequently in the same species, 14–20 metabolites and enzymes related to carbohydrates, proteins, and lipids were further examined, and the results revealed that the higher energy consumption of the liver during osmotic acclimation is mainly supplied by carbohydrates (Polakof et al., 2006). This was further supported by a recent study on the Senegalese sole (*Solea senegalensis*), a marine euryhaline teleost; plasma glucose levels were found to have increased following acclimation to an extreme (55 ppt) salinity for 2 wk (Arjona et al., 2007). Taken together, changes in energy metabolism during osmotic challenge reflect high hepatic glycogenolytic activity and a resultant elevation in the plasma glucose level (van der Boon et al., 1991; Wendelaar Bonga, 1997; Arjona et al., 2007). Indeed in Perry and Walsh's study (1989), isolated tilapia (*O. mossambicus*) gill cells oxidized both glucose and lactate greater than other metabolites (alanine or oleate). Apparently, most of those previous studies have addressed the major role of carbohydrate metabolism in metabolic reorganization during salinity acclimation (Woo and Murat, 1981; Nakano et al., 1998; Kelly and Woo, 1999; De Boeck et al., 2000).

The energy required by osmoregulatory organs in fish, including the gills and kidneys, is thought to be maintained by oxidation of glucose and lactate obtained from the circulation (Mommsen, 1984; Mommsen et al., 1985). Apparently, the liver is the central site involved in glucose turnover in fish as in other vertebrates. In addition to the levels of glucose and lactate, changes in glycogen contents are another indicator for monitoring carbohydrate mobilization in a specific organ or cell. Effects of environmental salinities on glycogen contents of the liver, gills, and/or other organs have been examined in several studies, although the results were inconsistent (Assem and Hanke, 1979; Nakano et al., 1998; Chang et al., 2007b; Tseng et al., 2007). Activities of GP, the rate-limiting enzyme for utilizing glycogen, have also been examined in the liver, gills, kidneys, and brain of gilthead sea bream (*S. auratus*) during the 2-wk SW acclimation (Sangiao-Alvarellos et al., 2005; Polakof et al., 2006). However, the physiological significance of glycogen mobilization in the main osmoregulatory organ, the gills, seems to have been overlooked until recently. Tseng et al. (2007) identified a novel GP isoform (tGPGG) and found its specific expression in a certain group of cells, glycogen-rich (GR) cells, which reside next to mitochondrion-rich cells (MR cells, the main ionocytes) in tilapia gills. Glycogen synthase, the rate-limiting step in the glycogen synthesis pathway (Garcia-Rocha et al., 2001), was also identified to be co-expressed in tilapia gill GR cells (Chang et al., 2007b). Salinity-dependent changes in mRNA and protein expressions, and/or activities of GP and GS (Tseng et al., 2007; Chang et al., 2007b), as well as the inhibitory effects of caffeine (an inhibitor of GP) on gill Na^+-K^+-ATPase activity *in vitro* and *in vivo* (Hwang and Lee, 2007) indicated modulation of glycogenesis and glycogenolysis in gill GR cells, which may provide energy to the neighboring MR cells to carry out ion transport during salinity challenges (Fig. 1). This spatial relationship in energy translocation between gill GR cells and MR cells has been well documented in

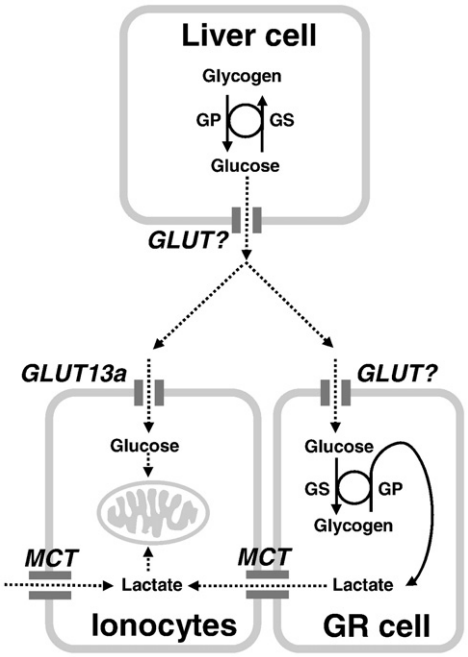

Fig. 1. Glycogen metabolism and the transport of the subsequent metabolites in liver and gill cells. Glycogenolysis and glycogenesis are conducted in both liver cells and gill glycogen-rich (GR) cells. Glucose from the degradation of the liver glycogen is transported via circulation system to gills, and is taken by gill ionocytes and GR cells via glucose transporter (GLUT) 13a and other GLUT isoform, respectively. Glucose is deposited as glycogen in GR cells, and lactate may be synthesized through glycogenolysis and transported via monocarboxylate transporter (MCT) into the neighboring ionocytes. In ionocytes, the glucose from liver and the lactate from GR cells are used as energy source for the operations of ion regulation mechanisms. Solid lines indicate the metabolism pathways, and dashed lines represent the transport pathways of metabolites. Question mark: unidentified transporter. GP, glycogen phosphorylase; GS. glycogen synthase.

mammal astrocytes and neurons (Pfeiffer-Guglielmi et al., 2003). In the rat brain, GP and glycogen mainly exist in astrocytes and astroglial cells, but never in neurons; during energy deprivation in the central nervous system, glycogen is degraded to lactate, which is shuttled from astrocytes to high-energy-requiring neurons (Ransom and Fern, 1997; Brown et al., 2003; Pfeiffer-Guglielmi et al., 2007).

Subsequent experiments by Chang et al. (2007b) further outlined the spatial and temporal partitioning of the energy supply for osmoregulation by gill MR cells during salinity acclimation. A model of carbohydrate metabolism related to osmoregulation in tilapia gills during acute exposure to salinity stress was proposed (Chang et al., 2007b; Hwang and Lee 2007): GR cells accumulate glycogen as a local carbohydrate reserve in gills, glycogenolysis in gill GR cells is initially activated to provide prompt energy for neighboring MR cells in order to trigger ion secretion mechanisms, and several hours later, liver cells begin to degrade their glycogen for the subsequent energy supply (Fig. 1).

During salinity challenges, additional energy is required not only to stimulate the activities of Na^+-K^+-ATPase and other transporters or enzymes in the gills, intestines, and other osmoregulatory organs (Kelly and Woo, 1999; Ando et al., 2003; Evans et al., 2005; Hwang and Lee 2007), but also for the costs as affected by other metabolic processes which respond to changes in salinity (Morgan and Iwama, 1991). Several lines of evidence support this notion. In the case of tilapia, the glycogen concentration (mg/g protein) in liver cells is much greater, by about ~300-fold, than that in gill GR cells, and SW transfer caused about 75% and 50% decreases in liver cells and gill GR cells, respectively (Chang et al., 2007b), indicating that the energy supply from glycogen in the liver also contributes to the energy requirements of other organs. In gilthead sea bream (*S. auratus*), the kidneys, another osmoregulatory organ, and brain, a non-osmoregulatory organ, both showed changes in glycogenolysis during salinity acclimation (Sangiao-Alvarellos et al., 2005, Polakof et al., 2006). These imply that like the gills, the kidneys and brain (or other organs) may also accumulate glycogen as a local carbohydrate reserve to provide prompt energy for their own functional regulation during acute salinity challenge. Our experiment provided further evidence to support this inference. In the tilapia brain, an acute salinity challenge induced immediate and dramatic activation of Na^+-K^+-ATPase activity, about 1.3- and 2.4-fold increases at 1 and 6 h post-transfer, respectively (Fig. 2A), and these were accompanied by rapid decreases in the brain GP protein level with a peak at 1–3 h post-transfer (Fig. 2B), and an evident decrease in that of GS (Fig. 2C). The increased brain Na^+-K^+-ATPase activity may be associated with regulation of intracellular ions or the activation of other neural activities during salinity challenge. Taken together, the liver is definitely the central carbohydrate reserve for the entire system, but the role of each organ in the local energy supply for emergency requirements during acute challenge by salinity or other stressors cannot be ignored.

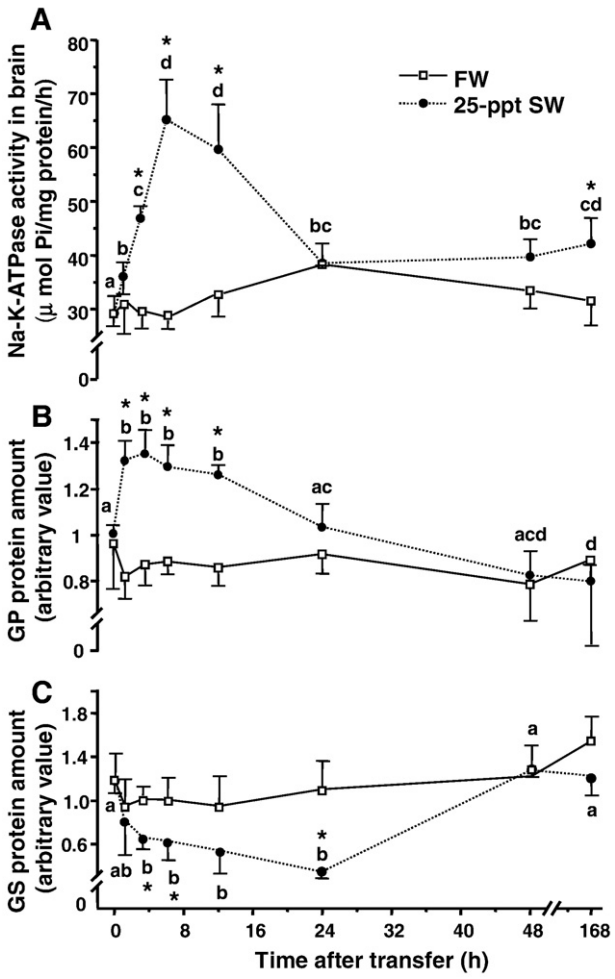

Fig. 2. Time-course changes in the brain's (A) Na^+–K^+-ATPase activity, (B) relative amounts of the glycogen phosphorylase (GP) protein, and (C) the glycogen synthase (GS) protein in tilapia (*Oreochromis mossambicus*) transferred from fresh water (FW) to 25 ppt seawater (SW). The relative protein amounts of GP and GS were quantified by comparing the immunoreactions by Western blotting (anti-human GP and anti-human GS antibodies) normalized to the intensities of actin. A: Brain Na^+–K^+-ATPase activity showed a rapid increase 1 h after transfer to SW; the increasing activity peaked 6 h post-transfer, and had returned to near the level of the FW control by 24 h post-transfer. B and C: Following changes in Na^+–K^+-ATPase activity, the brain's GP protein showed an increasing level while the GS protein decreased immediately post-transfer. GP and GS proteins had returned to near that of the control FW group by 24–48 h post-transfer. Data are presented as the mean ± S.D. (N=6). Two-way ANOVA was used for the statistical analysis. * Indicates a significant difference from the respective control in FW (p<0.05). Different letters indicate a significant difference (p<0.05) among sampling times for fish transferred to SW.

4. Transport of metabolites

In the processes of absorption, metabolism, and utilization, most nutrients and the subsequent metabolites have to be transported across cell membranes via various transporters. The expressions and functions of these transporters are upregulated to enhance the transport of nutrients and subsequent metabolites when an additional energy supply is required for compensatory physiological processes particularly in fluctuating environments. Thus, profiles of the expressions and functions of these transporters reflect changes in energy metabolism.

4.1. Fatty acid transport

Initially, it was believed that most digested products of lipids pass across the plasma membrane, composed of a phospholipid bilayer, of cells merely by simple diffusion. Nowadays, it is known that only a fraction of the fatty acids, long-chain fatty acids, can enter epithelial cells of small intestinal villi via the fatty acid transporter protein (FATP) (Hirsch et al., 1998; Stahl et al., 2001; Stahl, 2004). Some hormones and cytokines have been reported to influence FATP expression (Frohnert et al., 1999; Pohl et al., 2004). Insulin can induce FATP translocation in murine plasma membranes, thus resulting in an increase in LCFA uptake (Stahl et al., 2001; Fisher and Gertow, 2005). Altered fatty acid uptake due to impaired FATP function has been implicated in diseases such as insulin resistance and obesity in mammals (Kim et al., 2004; Pohl et al., 2004). Teleost plasma generally contains higher insulin compared to that of mammals (Moon, 2001). Partial sequences of FATP genes from puffer fish (*Fugu rubripes*) have been identified (CA330766). However, whether this protein family, solute carrier protein (SLC) 27 is involved in the uptake of long-chain fatty acids by cells still requires further investigation. Determining if these FATPs are upregulated to enhance the energy supply from lipids

during environmental challenge, including osmotic variances, also deserves study.

4.2. Amino acid transport

In enterocytes, the basic mechanisms of amino acid absorption are carrier-independent diffusion (for L-amino acid via a paracellular route), Na^+-independent facilitated transport (for BCAAs and aromatic amino acids), and Na^+-dependent active transport absorption (for most neutral amino acids) (Mailliard et al., 1995; Fan et al., 2001). On the other hand, the basolateral membrane of enterocytes contains additional transporters, such as aromatic amino acid transporter 1 (TAT1), which exports amino acids from cells into the blood independent of Na^+ (Ramadan et al., 2006).

In fish, proteinaceous materials can be absorbed as several forms. Di- and tri-peptides need not be completely hydrolyzed to free amino acids before they are absorbed by enterocytes because fish also possess a transport system for small peptides (Ash, 1985). In addition to small peptides, free amino acids or whole proteins can also be absorbed by fish enterocytes, indicating flexibility in protein uptake mechanisms (Smith, 1989). On the other hand, larger peptides and intact proteins can be absorbed into enterocytes via pinocytosis or can directly enter the bloodstream via a paracellular route (Jobling, 1995).

As described above, early investigations found that free amino acids are organic osmolytes involved in maintaining the cellular and plasma osmolarity of fish (Love, 1970). However, how these organic osmolytes are transported is still not conclusively known. Previously, the taurine transporter was identified and cloned from tilapia (Takeuchi et al., 2000). This is the leading investigation describing the taurine transporter in euryhaline fish. mRNA levels of this gene in the osmoregulatory organs of the gills, kidneys, and intestines were markedly upregulated during acclimation to 70% artificial SW (713 mOsm/kg H_2O) for 12 h, but then decreased back to a level close to that of the controls after 24 h (Takeuchi et al., 2000), indicating a short-term response by the compensatory processes of tilapia. Similar results were also noted in Atlantic salmon (*S. salar*) in response to hyperosmotic stress (Zarate and Terence, 2007). In mammals, there are other amino acid transporters belonging to the SLC7 and SLC6 protein families that are also involved in amino acid transport (Kleta et al., 2004; Ramadan et al., 2006; Fukuhara et al., 2007). It is unknown whether those protein families also exist in fish and whether they are used for amino acid absorption, or whether those transporters are also stimulated in order to enhance the transport of specific amino acids to provide additional energy or osmolytes to osmotically stressed fish.

4.3. Glucose transport

Glucose transport across cell membranes occurs mainly via a specific group of the SLC family, namely the facilitative Na^+-independent sugar transporters (of the GLUT family, SLC2A). At least 13 GLUT isoforms have been reported to occur in mammals. They possess multiple functional homologues and show tissue/cell-type specific expression patterns (Joost and Thorens, 2001; Joost et al., 2002). The mammalian SLC2 family isoforms can be further classified into three subclasses based on amino acid sequences and transport characteristics (Joost and Thorens, 2001; Joost et al., 2002); class I includes GLUTs 1–4, which are the best understood. Class II comprises the fructose transporter, GLUT5, and three recently described proteins, GLUT7, GLUT9, and GLUT11. Class III is comprised of five newly identified members, GLUT6, GLUT8, GLUT10, GLUT12, and proton myo-inositol transporter (HMIT).

Compared with studies of fatty acids and amino acids, those of glucose transport in fish have received considerable attention for several possible reasons. (1) Blood glucose levels are species-specific, ranging from <0.5 mmol/L in goldfish (Chavin and Young, 1970) to >50 mmol/L in Amazonian armoured catfishes (*Glyptoperichthyes gibbceps* and *Liposarcus pardalis*), (MacCormack et al., 2003), and environmental disturbances, like hypoxia or osmotic variations, can cause several multiples of change in fish blood glucose levels (Shoubridge and Hochachka, 1983; Sangiao-Alvarellos et al., 2005). (2) Elevated plasma glucose is thought to be an indicator of a stress response in fish (Wendelaar Bonga, 1997). (3) The phenomenon of glucose intolerance has been well documented in fish through the study of dietary carbohydrate contents (Moon, 2001; Díaz et al., 2007). Most fish studies to date focused on the GLUTs of class I. Immunoreactions with anti-mammalian GLUT1 antibodies were detected only in the brain and heart of Nile tilapia (*Oreochromis nilotica*) (Wright et al., 1988). Orthologues of the mammalian and avian GLUT1 have been cloned from rainbow trout (*O. mykiss*) (Teerijoki et al., 2000), common carp (*Cyprinus carpio*) (Teerijoki et al., 2001), and Atlantic cod (*G. morhua*) (Hall et al., 2004). Orthologues of the mammalian GLUT2 in rainbow trout (Krasnov et al., 2001), GLUT3 in grass carp (*Ctenopharyngodon idellus*) (Zhang et al., 2003), and GLUT4 in brown trout (*O. mykiss*) (Planas et al., 2000) and coho salmon (*Oncorhynchus kisutch*) (Capilla et al., 2004) have also been sequenced, and a part of them have further been functionally analyzed. However, it is unclear whether other GLUT isoforms exist in fish, and little is known about the GLUTs and their functional roles in energy supply for osmoregulatory organs and ionocytes.

Using a functional genomic approach, our preliminary experiments (Y. C. Tseng, J. R. Lee, S. J. Lee, and P. P. Hwang; unpublished data) identified members of the GLUT family in zebrafish (*Danio rerio*) on a large scale. According to a Neighbor-joining (NJ) phylogenetic analysis, zebrafish GLUTs were clustered into three distinct groups as were their mammalian counterparts (Fig. 3). A reverse-transcriptase polymerase chain reaction (RT-PCR) analysis showed tissue-specific expression patterns of zGLUT isoforms (Fig. 4), and gills, the main osmoregulatory organ, expressed several isoforms including zGLUT1a, 3, 6, 8, 10, 12, and 13a (Fig. 4). Among them, zGLUT13a (EU499348), a novel isoform, is the first one to be identified which is specifically expressed in larval gills and skin by whole-mount *in situ* hybridization (Fig. 5), and the "pepper-like" expression pattern is similar to that of ionocytes in larval gill/skin before the full development of gills, as

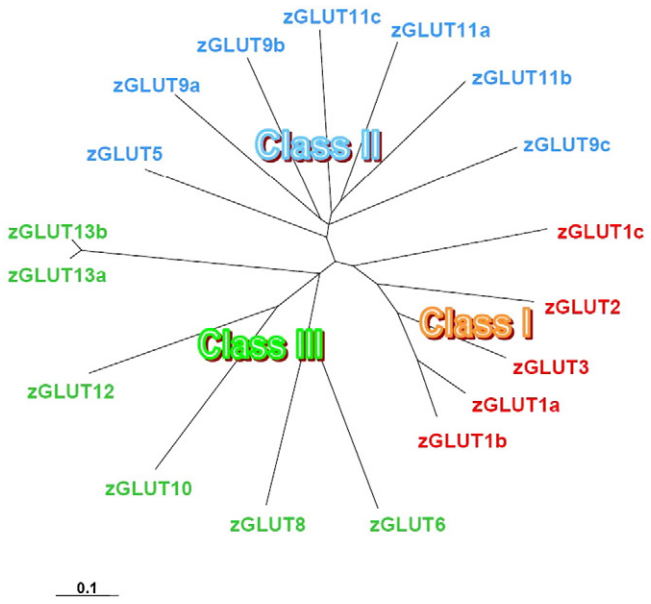

Fig. 3. Radial tree showing multiple alignment of all members of the zebrafish (*Danio rerio*) glucose transporter (zGLUT) family that have been cloned and sequenced. The tree was constructed using the ClustalW program from the DNA Data Bank of Japan (DDBJ; http://www.ddbj.nig.ac.jp/). The MEGA program (version 3.1.) was used for the Neighbor-joining (NJ) analysis with 10,000 bootstrap replicates. Three subclasses were clearly distinguishable in the zGLUT family.

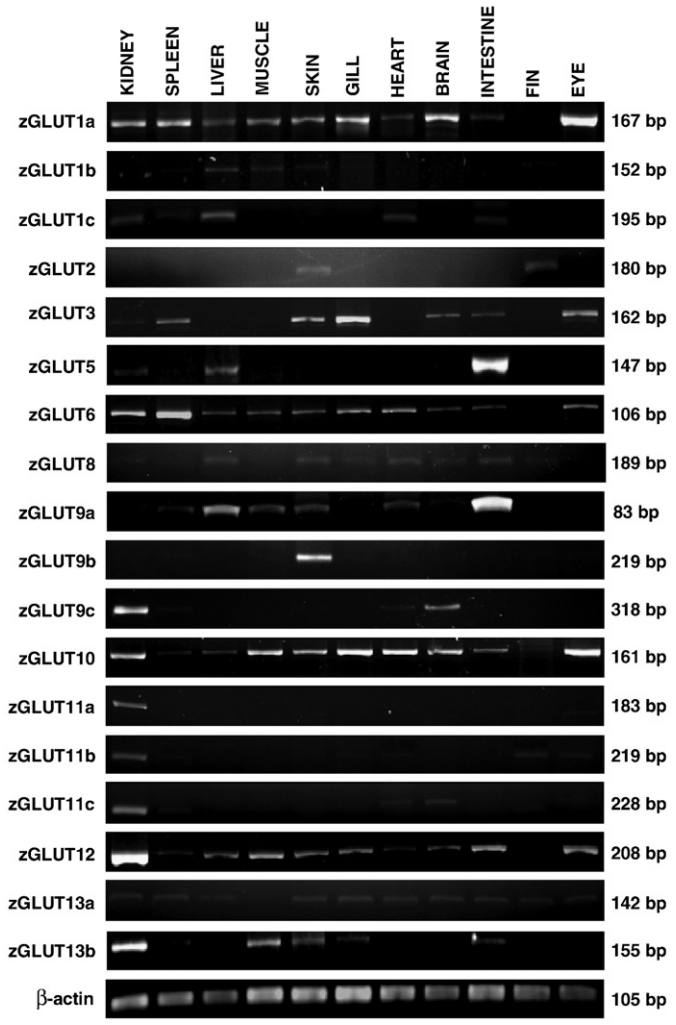

Fig. 4. RT-PCR analysis of the zebrafish (*Danio rerio*) glucose transporters (zGLUTs) in various tissues of zebrafish with isoform-specific primer sets. β-actin was used as the internal control. Each zGLUT showed a tissue-specific expression pattern.

reported in previous studies on zebrafish ionocytes (Pan et al., 2005; Lin et al., 2006; Esaki et al., 2007; Hsiao et al., 2007). Our preliminary experiments on further functional analysis provided the 1st cue for the role of zGLUT13a in the energy supply needed for the functioning of gill/skin ionocytes. *In vivo* loss-of-functional analyses with specific morpholino oligonucleotides found that knock down of zGLUT13a not only produced a lower density and smaller size of ionocytes apical openings (ConA as a marker), but also impaired the function of Na^+ uptake which was detected with either a Na^+-dependent fluorescent reagent (Na green) or the $^{24}Na^+$ radioisotope (Y. C. Tseng, S. J. Lee, J. R. Lee, S. Hirose and P. P. Hwang; unpublished data). In subsequent experiments, acclimation to low-Na^+ artificial FW, which can stimulate Na^+ uptake and expression of Na^+/H^+ exchanger 3b in gill/skin ionocytes (Yan et al., 2007; Lin et al., 2008), was also found to induce a 4-fold stimulation of zGLUT13a expression (Fig. 6). This implies increased transport of glucose for an additional energy supply for enhancing the Na^+ uptake function of ionocytes during acclimation to Na^+-deficient FW. These preliminary results are of physiological significance by providing convincing molecular physiological evidence to link ionic regulation and its energy supply in fish ionocytes (Fig. 1).

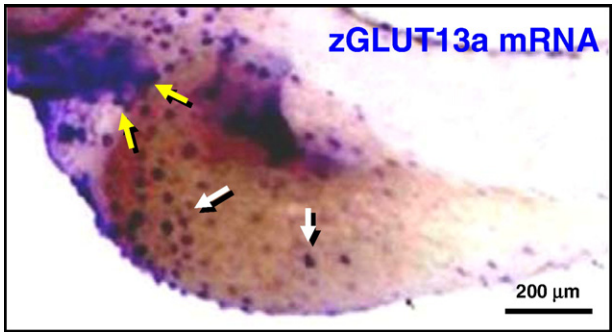

Fig. 5. Whole-mount *in situ* hybridization of zebrafish (*Danio rerio*) glucose transporter 13a (zGLUT13a) in zebrafish larvae. Using a specific RNA probe for zGLUT13a (bankit1064924; nt 1053–1320) the mRNA signals were predominately found in gills (yellow arrow) and yolk-sac skin (white arrow) of 3-d post-fertilization zebrafish.

4.4. Monocarboxylate transport

Lactate was also found to be another energy substrate of cells, especially in the central nervous system and myofibrils (Porras et al., 2004; Bergersen, 2007). In a condition of hypoglycemia, the brain can utilize monocarboxylates as substitute fuels. Lactate is known to preferentially be used by neurons, and is transported from astrocytes to neurons via the monocarboxylate transporter (MCT) (Pellerin, 2005; Bouzier-Sore et al., 2006). Recently, MCTs have been proposed as playing a central role in the mechanisms of emergency energy translocation, by the current hypothesis of an "astrocyte-neuron lactate shuttle", in the mammalian brain (Pellerin, 2005). In the mammalian SLC16 protein family, only MCT1, 2, 3, and 4 have been characterized by processing the ability to transport monocarboxylates with different affinities (K_m), and tissue/cell-type specific expression patterns (Manning Fox et al., 2000; Philp et al., 2001; Bergersen et al., 2006; Bergersen, 2007; Chiry et al., 2006; Hashimoto et al., 2007; Zhang et al., 2007).

Fish also appear to use lactate as an energy substrate during salinity acclimation based on previous studies of changes in plasma lactate levels, as well as lactate contents and lactate dehydrogenase expression/activity in osmoregulatory organs (Vijayan et al., 1996, Sangiao-Alvarellos et al., 2003b, 2005; Polakof et al., 2006; Tseng et al. in press). However, nothing is known about the transport of lactate in fish. From genetic databases, MCT1, 2, 3 and 4 were identified in zebrafish (zMCT1: NM_200085; zMCT2: XM_684122; zMCT3: XM_681038; zMCT4: NM_212708). Our preliminary data showed that specific types of MCT are expressed in zebrafish gill ionocytes and GR cells (refer to Section 3.3) (Y. C. Tseng, Z. J. Kao, S. T. Liu, and P. P. Hwang; unpublished data), indicating the possibility that MCTs may transport lactate between different types of gill cells (Fig. 1). This opens an unexplored issue that is important for elucidating the role of

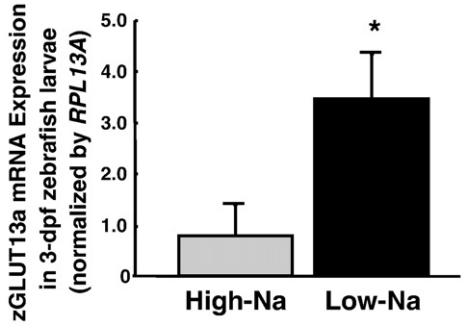

Fig. 6. Comparison of zebrafish (*Danio rerio*) glucose transporter 13a (zGLUT13a) transcripts (quantitative real-time PCR) in 3-d post-fertilization zebrafish larvae acclimated to high-Na (Na^+: 10 meq/L) and low-Na (0.04 meq/L) artificial fresh water (FW) for 1 week. zGLUT13a was upregulated in low-Na larvae compared with that in high-Na ones. Data are presented as the mean ± S.D. ($N=4$). * Indicates a significant difference (Student's *t*-test, $p<0.05$).

Table 1
List of upregulated genes in *Dicentrarchus labrax* during salinity challenge

Gene name[a]	Accession no.[a]	Tissue[a]	Medium[a]	Metabolic process
Cytochrome *c* oxidase subunit Va	CX660594	Intestines	FW	Mitochondrial electron transport chain
Cytochrome *c* oxidase subunit III	CX660445	Intestines	FW	Mitochondrial electron transport chain
Cytochrome *c* oxidase subunit I	CX660601	Intestines	FW	Mitochondrial electron transport chain
Cytochrome *b*	CAA57262	Intestines	FW	Mitochondrial electron transport chain
Succinate dehydrogenase C	CX660602	Intestines	FW	Mitochondrial electron transport chain
Fructose-biphosphate aldolase B	CX660600	Intestines	FW	Glycolysis
Glyceraldehyde-3-phosphate dehydrogenase	CX660611	Intestines	FW	Glycolysis
Alpha-aspartyl dipeptidase	CX660615	Intestines	FW	Dipeptide hydrolysis
Very-long-chain acyl-coA synthetase	CX660598	Intestines	FW	Fatty acid catabolism
Intestinal fatty acid-binding protein	CX660610	Intestines	FW	Lipid uptake and sensing
Soluble epoxide hydrolase	CX660617	Intestines	FW	Fatty acid catabolism
C1q-like adipose-specific protein	CX660618	Intestines	FW	Fatty acid catabolism
Isocitrate dehydrogenase	CX660439	Gills	FW	TCA cycle
ATP synthase c-subunit	CX660449	Gills	FW	Mitochondrial electron transport chain
Ecto-ATPase	CX660457	Gills	FW	ATP hydrolysis
Arginase	CX660463	Gills	FW	Arginine catabolism/ urea cycle
Ornithine decarboxylase antizyme small isoform	CX660465	Gills	FW	Polyamine synthesis
Cytochrome *c* oxidase subunit Vb	CX660885	Intestines	SW	Mitochondrial electron transport chain
Glucosamine-6-phosphate deaminase	CX660894	Intestines	SW	Glycolysis
ATP synthase FO subunit 6	CX660904	Intestines	SW	Mitochondrial electron transport chain
Solute carrier family 15	CX660890	Intestines	SW	Oligopeptide transport
Proteasome subunit beta 3	CX660895	Intestines	SW	Peptide cleavage
Dipeptidylpeptidase 4b	CX660897	Intestines	SW	Peptide cleavage/ glucose metabolism
Aminopeptidase N	CX660898	Intestines	SW	Peptide cleavage
Cystatin	CX660899	Intestines	SW	Proteolysis
Apolipoprotein B	CX660896	Intestines	SW	Lipid uptake
Mitochondrial ATP synthase gamma subunit	CX660761	Gills	SW	Mitochondrial electron transport chain
Cytochrome *c* oxidase subunit III	CX660445	Gills	SW	Mitochondrial electron transport chain
Epidermis-specific serine protease	CX660751	Gills	SW	Proteolysis
Protein disulfide isomerase-related protein	CX660755	Gills	SW	Proteolysis
Rhomboid, veinlet-like 2	CX660757	Gills	SW	Proteolysis

[a] Data from Boutet et al. (2006); FW, fresh water; SW, seawater.

lactate in emergent energy supplies for fish gill cells during acute challenge with salinity variances.

5. Functional genomic studies of energy metabolism

Global analysis of mRNA abundance by a complementary (c)DNA microarray or other functional genomic approaches is useful and popular for finding genes that are differentially expressed between groups with different treatments, thus providing a functional genomic view to identify novel genes, new functions of known genes, and novel mechanisms relevant to the treatments. An "environmental genomic" method has been developed as a powerful approach to explore interactions between the whole genomes of organisms and the environment (Cossins and Crawford, 2005), and it has been used to examine the complex programs of fish gene expressions associated with various stressors, including hypoxia (Ton et al., 2003), low temperatures (Gracey et al., 2004), pollutants (Koskinen et al., 2004), and salinity levels (Kalujnaia et al., 2007a). Many differentially expressed genes are related to energy metabolism in various organs of fish, indicating that functional genomics also provide an approach for examining changes in energetic mobilization during challenges with environmental stressors.

Recently, several studies have used functional genomic approaches to investigate salinity-dependent genes that are differentially expressed in various organs between SW and FW fish. Fiol and Kultz (2005) used suppression subtractive hybridization in tilapia gills to identify osmotic stress transcription factor 1 and transcription factor II B, which are rapidly and transiently induced during hyperosmotic stress. Using suppression subtractive hybridization and/or combined with a cDNA microarray analysis, other studies reported the profiles of gene transcripts affected by environmental salinities in the brain, gills, intestines, and kidneys of European eel (*Anguilla anguilla*) and sea bass

Table 2
List of upregulated genes in *Anguilla anguilla* during salinity challenge

Gene name[a]	Accession no.[a]	Tissue[a]	Medium[a]	Metabolic process
Maltase-glucoamylase, intestinal	XP_422811.1	Intestines	FW	Polysaccharide metabolism
Sucrase-isomaltase	XP_001236721	Intestines	FW	Polysaccharide metabolism
Cysteine dioxygenase type I	AAH59531.1	Intestines	FW	Amino acid catabolism
Solute carrier family 15 oligopeptide transporter	AAQ65244.1	Intestines	FW	Peptide transportation
Cathepsin B	AAQ97764.1	Intestines	FW	Proteolysis
Serine protease inhibitor	AAT68086.1	Intestines	FW	Proteolysis
Fatty acid-binding protein 6	NP_058794.1	Intestines	FW	Lipid uptake
Apolipoprotein A-I	BAE45335.1	Intestines	FW	Lipid uptake
14-kDa apolipoprotein	AB046209.1	Intestines	FW	Lipid uptake
Apolipoprotein B-100 precursor	XP_694827.1	Intestines	FW	Lipid uptake
Arachidonate l ipoxygenase 3	XP_546605.2	Gills	FW	Dioxygenation/ polyunsaturated fatty acid catabolism
NADH dehydrogenase subunit 2	YP_164025.1	Kidneys	FW	Mitochondrial electron transport chain
Cytochrome *c* oxidase subunit I	BAB39714.1	Kidneys	FW	Mitochondrial electron transport chain
Apolipoprotein A-I	BAE45335.1	Kidneys	FW	Lipid-binding proteins
Cytochrome *c* oxidase subunit I	BAB39714.1	Intestines	SW	Mitochondrial electron transport chain
Enolase 1 (alpha)	AAQ97775.1	Intestines	SW	Glycolysis
Ornithine decarboxylase antizyme, large isoform	AAP82035.1	Intestines	SW	Polyamine synthesis
Arachidonate lipoxygenase 3	XP_546605.2	Gills	SW	Dioxygenation/ polyunsaturated fatty acid catabolism
Cysteine dioxygenase, type I	AAH59531.1	Kidneys	SW	Amino acid catabolism
Aminopeptidase N	NP_999442	Kidneys	SW	Amino acid metabolism

[a] Data from Kalujnaia et al. (2007b); FW, fresh water; SW, seawater.

(*Dicentrarchus labrax*) (Boutet et al., 2006; Kalujnaia et al., 2007a,b); they addressed the gene expressions of known osmoregulation-related transporters, enzymes, and hormones as well as those related to other cellular events or physiological processes. In the lists of differentially expressed genes of those studies, there are many genes that are relevant to energy metabolism according to the authors' annotations (Boutet et al., 2006; Kalujnaia et al., 2007b); however, the physiological significances of those energy metabolism-related genes appear to have been overlooked. As shown in Tables 1 and 2, those genes are associated with many metabolic processes, including the mitochondrion electron transport chain, the TCA cycle, glycolysis, polysaccharide metabolism, amino acid catabolism, peptide cleavage and transport, proteolysis, fatty acid catabolism, lipid binding and uptake, and polyunsaturated fatty acid catabolism. The regulation of these genes for different metabolic processes was found to be dependent on the salinity, duration of acclimation, the organ, and fish species. Tables 1 and 2 also show several interesting phenomena: (1) more genes were upregulated in FW fish than in SW ones; (2) more genes were upregulated in the intestines (in either FW or SW) than in the kidneys and gills; and (3) in both species (sea bass and European eel), many genes related to lipid metabolism were upregulated in the intestines of fish in FW. More importantly, some of those genes have never been mentioned in previous studies on energy metabolism related to salinity (SW or FW) acclimation (refer to the other sections of the present paper), and thus provide new windows to approach novel pathways, which require further investigation in the future.

6. Conclusions and perspectives

A sufficient and timely energy supply is a prerequisite for the operation of iono- and osmoregulatory mechanisms in fish. Measurements of whole-fish or isolated-gill (or other organs) oxygen consumption, although with some constraints in the methodologies, have provided important data to demonstrate the regulation of energy supply during acclimation to different osmotic environments, and such regulation depends on the species, the situation of acclimation or acclimatization, and life habits. Data of the O:N ratio indicate the reorganization and utilization of different metabolic substrates as the energy supply during acclimation to fluctuating salinities. Carbohydrate metabolism appears to play a major role in the energy supply for iono- and osmoregulation, and the liver is the major source supplying carbohydrate metabolites to osmoregulatory organs. Compared with carbohydrates, the roles of lipids and proteins remain largely unclear, although some studies have discussed non-esterified fatty acids and some amino acids. Recently, energy metabolite translocation, known to occur between mammalian neurons and astrocytes, has been also found between fish gill ionocytes and neighboring glycogen-rich cells (Fig. 1). Now the physiological significance of a local energy supply for gill ion regulatory mechanisms can be further explored. Moreover, a new window has opened for examining spatial and temporal relationships between the liver and other osmoregulatory and non-osmoregulatory organs in partitioning the energy supply for ion regulatory mechanisms during salinity challenges (Fig. 1). The transport of metabolites should be upregulated to enhance the movement of nutrients and subsequent metabolites when additional energy supplies are required for compensatory physiological processes. zGLUT13a, a novel glucose transporter, has been found to specifically be expressed and function in gill ionocytes. The identification and functional analysis of the transporters of fatty acids, amino acids, and monocarboxylates remain a frontier for exploration in the future.

Having the benefit of advanced techniques of molecular/cellular biology, functional genomics, and model animals, much recent progress has contributed to our understanding of the iono- and osmoregulatory mechanisms in fish, by not only elucidating those mechanisms at the cellular and molecular levels but also opening several new windows for relevant issues (Hwang and Lee, 2007). Therefore, it is a good time to take advantage of these advanced methodologies to look at energy metabolism processes that are relevant to a particular cell type (e.g., gill ionocytes). Functional genomics may provide another powerful approach to explore new metabolic pathways related to fish ion regulation, since recent studies using microarray analyses have reported some candidate genes.

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