Summary of approaches to modeling hypoxia in *M. menidia* DEB

11/1/23

* Additional notes on how IGFBP-1 works
  + Hypoxia 🡪 HIF-1 pathway activated 🡪 HIF-1 binds to DNA and activates target gene expression 🡪 IGFBP-1 expression increases 🡪 IGFBP-1 binds insulin-like growth factors (IGFs) 🡪 IGFs can not bind to their receptors and thus action (signaling) on cell growth is inhibited
  + IGFs manage the effect of growth hormones in controlling tissue and bone growth.
    - They are called “insulin-like” because they are similar in molecular structure to insulin.
  + IGF stimulates mitogenesis, differentiation, and survival, so increasing IGFBPs (of which there are 7 in total) inhibits these processes.
    - Cell adhesion, migration, and apoptosis are some processes influenced by IGFBP-1.
    - In addition to hypoxia, IGFBP-1 can respond to food deprivation, malnutrition, stress, and chronic disease in a variety of vertebrates.
  + Overexpression of IGFBP-1 reduced embryonic growth in zebrafish and mice, and is associated with intrauterine growth restriction in humans.
  + Knockdown of IGFBP-1 *partially* abrogates the effect of hypoxia on growth and development, suggesting it is not solely responsible for effects.
    - There are a few other genes related to cell division and protein synthesis that are shown to be regulated by hypoxia in zebrafish (demonstrated in Ton et al., 2003).
  + Similarly, overexpression of IGFBP-1 in normoxia reduces growth (see figure below for differences in growth from Kajimura et al., 2005).
  + **IGFBP-1 inhibits IGF-stimulated proliferation of cultured zebrafish embryonic cells.** Adding just IGF to the cells leads to cell proliferation (compared to no IGF), and adding IGFBP-1 reduces the cell proliferation if the concentration of IGFBP-1 > IGF. See figure below.
  + Regarding whether this mechanism could reduce assimilation by reducing energy demand for growth, it is still hard to tell. Is energy flow generally dictated by demand, where the flow would decrease if the energy is not being used as quickly for cell division and differentiation? Or is it more of a “top-down” process where the flux proceeds at the same rate regardless of what the cells are doing with the energy?
    - A quote from Kajimura et al. (2005) implies that the adaptive advantage of this pathway is to save energy from being directed to growth during stressful conditions: “Because these stressful and catabolic conditions lead to adaptive changes in metabolic reorganization, such as the activation of the anaerobic ATP-generating pathway (glycolysis), the adaptive significance of the IGFBP-1 up-regulation may be to divert important energy resources away from growth and development toward those metabolic processes essential for survival.”
    - If it was still being directed but wasted there would be little advantage or reason for it to persist in evolution across so many species (although simply keeping the body size smaller would still be helpful under limited oxygen or food conditions).

A graph of a cell formation

Description automatically generated with medium confidence

Panel from Figure 5 in Kajimura et al. (2005) showing cell proliferation rates as a percentage of the control level. The first two bars are control cell proliferation with no IGFBP-1 (shortened to BP-1 here) or IGF, or with just IGFBP-1, showing that adding IGFBP-1 in the absence of IGF has no effect. The following bars show the effect of just adding 100 ng/ml of IGF, which dramatically increases cell proliferation up to 600% of the control cells. The next set of bars shows how adding IGFBP-1 reduces the cell proliferation by inhibiting the action of IGF. The last two sets of bars show that the more IGF there is, the less of an inhibitory effect IGFBP-1 has on cell proliferation. The white bars are IGF-1 and hashed bars are IGF-2, the two types of IGF.

10/26/23

**Hypoxia-inducible factors (Hif) and IGFBP-1 expression**

* Hif proteins control the hypoxic response and are crucial for proper development and organ differentiation in the embryo and larval stages.
* Hifs help fish tolerate hypoxia, so not necessarily a “damage” situation, but there could be tradeoffs in the pathways they activate.
* One gene whose expression is controlled by Hif is insulin-like growth factor binding protein 1 (IGFBP-1). Kajimura et al. (2005) studied this in zebrafish.
  + This protein binds insulin-like growth factors, and increasing it reduces growth.
  + Hypoxia (0.6 mg/L compared to 6.5 mg/L normoxia) strongly increased the mRNA expression and protein levels of IGFBP-1 in zebrafish embryos (24-72 hours post fertilization) at 28.5°C.
  + Growth is reduced: after 24 hours in hypoxia the embryos’ body length was ~0.5 mm smaller than those in normoxia.
  + Development was delayed, based on angle between head and trunk, markers of heart development, and markers of skeletal development.
  + Only two oxygen levels were used, so this cannot tell us if it is a linear or nonlinear response.
  + DEB mechanism impacted: assimilation rate and/or conversion efficiency.
    - Hypoxia causes reduced size at hatching, delayed hatching, greater mortality at hatching. This mechanism could explain all of these.
    - Energy to growth is reduced, but maintenance needs still exist so yolk would be depleted slowly and run out when embryo is at a smaller size.
* It helps the fish by reducing oxygen needs
* More species:
  + A study looked at IGFBP-1 gene expression during embryonic development alongside growth in blunt snout bream, but did not expose the embryos to hypoxic treatment concurrently – they only sought to demonstrate that increased IGFBP-1 leads to reduced growth (Tian et al., 2014).
  + This study correlated IGFBP-1 with hypoxia-induced growth reduction and developmental defects in grass carp embryos, where hypoxia is 1 mg/L compared to 7 mg/L normoxia (Sun et al., 2011).
* Notes from meeting Oct. 27:
  + No maturation in model, 1-k goes nowhere, but hatch when yolk is zero.
    - Development variable using maturity? Prefer not to go there
  + Connecting to model: two fluxes that could be cause of reduced growth, no implication for change in maintenance.
  + Does this suppression of growth control assimilation? Does this gene control assimilation or growth?
  + Not just as a discussion point but also better justification methods, parameters we tested
  + Know from toxicology work that purely using aic can be misleading
    - Erik Muller paper looking at effect of mercury on mussel larvae and oyster larvae growth

**Lactate concentration and enzyme activity of lactate dehydrogenase**

* Lactate concentration – lactate accumulation could cause damage, as well as reducing internal pH and thereby reducing hemoglobin affinity for oxygen.
  + A study exposing zebrafish offspring to mild hypoxia (4.3 mg/L compared to 7.5 mg/L normoxia) found no differences in lactate concentration until 30 days post hatching (Barrionuevo et al., 2010).
  + A review paper (Wieser, 1995) says that the capacity for anaerobic glycolysis develops gradually after termination of the yolk sac stage, citing a thesis (Finn 1994) that found very little lactate is produced in embryos and yolk sac larvae of marine fish.
    - This review mentioned one exception: lactate peaks early in embryonic development of Atlantic cod (Finn 1995). No growth, but maybe in Part II?
  + Killifish in diapause and an amphibious fish also appear to be exceptions in which evidence was found of anaerobic glycolysis in embryo stage, but these are unusual circumstances that do not apply to *Menidia*.
  + Another review paper (Nilsson and Östlund-Nilsson, 2008) says small fishes reach lethal levels of anaerobic end-products (which are mainly lactate) much faster than larger fishes because of their higher mass-specific metabolic rate.
  + Embryos of Arctic char supply energy at 11-23% the aerobic rate using anaerobic pathways (Gnaiger, 1979; Gnaiger et al., 1981 – as summarized in Rombough, 1988, because I couldn’t find the papers)
* Lactate dehydrogenase enzyme activity
  + LDH catalyzes the conversion of pyruvate (product of glycolysis) into lactate in the absence of oxygen, and in the liver it converts lactate back into glucose in the Cori cycle.
  + Gene expression of lactate dehydrogenase, an enzyme involved in anaerobic glycolysis, increased during hypoxia (5% oxygen) in zebrafish embryos exposed at 48 hours post fertilization (Ton et al., 2003).
  + Similarly, lactate dehydrogenase expression increased in medaka embryos exposed to hypoxia (4, 2, or 0.5 kPa, compared to 18 kPa normoxia) for 24-48 hours (Wawrowski et al., 2011).

**Metabolomics**

* Not much existing work on metabolomics responses to hypoxia in fish, and virtually none on early life stages.
  + The killifish *Austrofundulus limnaeus* that has an embryonic diapause (same species mentioned above) is one exception. A metabolomics analysis on them found that embryos tolerate anoxia through accumulation of lactate (as well as alanine and succinate, to a lesser degree) and large quantities of gamma-aminobutyrate (GABA), neural protector (Podrabsky et al., 2007).

**Synthesizing Unit**

* Generalized form of enzymes that follow enzyme kinetics.
  + In this case, enzymes that convert oxygen and food into assimilate.
    - Is the yolk food or assimilate in this relationship? I would think food because it already exists, whereas assimilates are the product that we need oxygen to get.
  + If oxygen is limiting there is less synthesis, but as oxygen gets higher the reaction rate levels off (Michaelis-Menten kinetics).
  + Do we assume a fixed flux of assimilates and variable oxygen levels? Or that there is always excess assimilates and oxygen is limiting?
* In DEB they use fluxes (not concentrations).
  + The oxygen concentrations would have to be changed to fluxes?
  + Oxygen consumption rate can be used.
  + Arrival flux is proportional to density in spatially homogeneous environments.
  + Uses an analogy that if the enzyme is the individual and the product is the reserve, the transformation rate is like the functional response. As density increases the reaction rate slows down, similar to handling time of food by a predator.
* Processing can be sequential or parallel
* Compounds can be complementary or substitutable
* This could be useful because then it doesn’t matter which enzyme we are talking about (LDH or something else), sort of bypasses damage variable.
* At high concentrations of substrate the equation simplifies to Michaelis-Menten kinetics: JC = JCm(1+xA-1)-1
* Does the Rejection Unit matter in this context?
* Notes from meeting Oct. 27:
  + Inhibitory effect on SU
  + Synthesizing unit – effect of limitation of one substrate (oxygen) gets at opposite end of energy flow from igfbp-1
  + Change formula of correction factor
  + Just one branch is oxygen limited? Because oxygen consumption doesn’t decrease with these levels of hypoxia
  + Upregulation of gene responsible for enzyme makes more of the enzyme, overall rate goes faster without kinetics increasing (each individual enzyme protein doing same rate as before)
  + Depends what happens to resource (food/yolk) – is it wasted or available for later?
  + Gets messy if dealing with two substrates and inhibition
  + Roger will work on figuring out which way we would use, and I will find more details on igfbp-1
  + If inhibitory SU is good enough
  + Roger is unavailable starting nov 13, going to Scotland Friday after thanksgiving, could look at a draft after dec 11,

**References**

Barrionuevo et al., 2010. Aerobic and anaerobic metabolism for the zebrafish, *Danio rerio*, reared under normoxic and hypoxic conditions and exposed to acute hypoxia during development. *Braz. J. Biol.*, 70: 425-434.

Finn, 1994. Physiological energetics of developing marine fish embryos and larvae. Thesis, University of Bergen, Norway.

Finn et al., 1995. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). I. Respiration and nitrogen metabolism. *Marine Biology*, 124: 355-369.

Kajimura et al., 2005. Insulin-like growth factor-binding protein-1 (IGFBP-1) mediates hypoxia induced embryonic growth and developmental retardation. *PNAS*, 102: 1240-1245.

Nilsson and Östlund-Nilsson, 2008. Does size matter for hypoxia tolerance in fish? *Biol. Rev.*, 83: 173-189.

Podrabsky et al. 2007. Extreme anoxia tolerance in embryos of the annual killifish *Austrofundulus limnaeus*: insights from a metabolomics analysis. *J. Exp. Biol.*, 210: 2253-2266.

Rombough, 1988. Respiratory gas exchange, aerobic metabolism, and effects of hypoxia during early life. In *Fish Physiology, Vol. 11, The Physiology of Developing Fish, Part A: Eggs and Larvae* (ed. W. S. Hoar and D. J. Randall), pp. 59-162. San Diego: Academic Press.

Sun et al., 2011. IGF binding protein 1 is correlated with hypoxia-induced growth reduce and developmental defects in grass carp (*Ctenopharyngodon idellus*) embryos. *General and Comparative Endocrinology*, 172: 409-415.

Tian et al., 2014. Molecular cloning and function analysis of insulin-like growth factor-binding protein 1a in blunt snout bream (*Megalobrama amblycephala*). *Zoological Research*, 35: 300-306.

Ton et al., 2003. Gene expression profile of zebrafish exposed to hypoxia during development. *Physiological Genomics*, 13: 97-106.

Wawrowski et al., 2011. Changes of globin expression in the Japanese medaka (*Oryzias latipes*) in response to acute and chronic hypoxia. *Journal of Comparative Physiology B*, 181: 199-208.

Wieser, 1995. Energetics of fish larvae, the smallest vertebrate. *Acta. Physiol. Scanda.*, 154: 279-290.