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Glutathione redox dynamics and expression of glutathione-related genes in the developing embryo

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ABSTRACT

Embryonic development involves dramatic changes in cell proliferation and differentiation that must be highly coordinated and tightly regulated. Cellular redox balance is critical for cell fate decisions, but it is susceptible to disruption by endogenous and exogenous sources of oxidative stress. The most abundant endogenous nonprotein antioxidant defense molecule is the tripeptide glutathione (γ-glutamylcysteinylglycine, GSH), but the ontogeny of GSH concentration and redox state during early life stages is poorly understood. Here, we describe the GSH redox dynamics during embryonic and early larval development (0–5 days postfertilization) in the zebrafish (*Danio rerio*), a model vertebrate embryo. We measured reduced and oxidized glutathione using HPLC and calculated the whole embryo total glutathione (GSH_T) concentrations and redox potentials (E_h) over 0–120 h of zebrafish development (including mature oocytes, fertilization, midblastula transition, gastrulation, somitogenesis, pharyngula, prehatch embryos, and hatched eleutheroembryos). GSH_T concentration doubled between 12 h postfertilization (hpf) and hatching. The GSH E_h increased, becoming more oxidizing during the first 12 h, and then oscillated around −190 mV through organogenesis, followed by a rapid change, associated with hatching, to a more negative (more reducing) E_h (−220 mV). After hatching, E_h stabilized and remained steady through 120 hpf. The dynamic changes in GSH redox status and concentration defined discrete windows of development: primary organogenesis, organ differentiation, and larval growth. We identified the set of zebrafish genes involved in the synthesis, utilization, and recycling of GSH, including several novel paralogs, and measured how expression of these genes changes during development. Ontogenic changes in the expression of GSH-related genes support the hypothesis that GSH redox state is tightly regulated early in development. This study provides a foundation for understanding the redox regulation of developmental signaling and investigating the effects of oxidative stress during embryogenesis.

Embryonic development involves precisely orchestrated events and processes including changes in cellular proliferation, differentiation, and left–right asymmetry that are dependent on redox signaling and intracellular redox potentials (E_h) [1–6]. Reactive oxygen species (ROS)[1] such as hydrogen peroxide, superoxide anion, and hydroxyl radicals are produced endogenously via respiration and oxygenating enzymes, such as NADH oxidases, fatty acyl-CoA oxidase, xanthine oxidase, cyclooxygenases and lipoxygenases, and cytochrome P450s [2,5–8]. ROS play important roles in normal embryonic development, with functions in signal transduction, cell-fate decisions, and apoptosis [6,9–12]. However, conditions such as diabetic embryopathy, preeclampsia, intrauterine growth restriction, or obesity can lead to altered embryo–fetal ROS levels, disrupting ROS-dependent signaling or causing damage to cellular macromolecules [7,13,14]. In addition, embryonic exposure to many chemicals, including a variety of drugs and environmental contaminants, can generate ROS or reactive intermediates and cause perturbations in cellular redox status [15–18]. Oxidative stress, defined as a disruption of redox signaling and control [19], contributes to the mode of action of numerous teratogens, including thalidomide, phenytoin, valproic acid, methamphetamine, ethanol,

Abbreviations: ROS, reactive oxygen species; GSH, reduced glutathione; Gcl, glutamate–cysteine ligase; Gclc, glutamate–cysteine ligase catalytic subunit; Gclm, glutamate–cysteine ligase modifier subunit; Gss, glutathione synthetase; GSSG, glutathione disulfide; PCA, perchloric acid.

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polycyclic aromatic hydrocarbons, methyl mercury, lead, cadmium, and paraquat [20–27]. Embryonic oxidative stress also may have delayed effects including carcinogenesis, cardiovascular dysfunction, and insulin resistance [28–30]. Thus, exposure to chemicals or other stressors that modulate intracellular redox potentials and cause oxidant-induced disruption of signaling during embryonic development in vertebrates is a significant concern. A variety of inherent antioxidant defenses have evolved to protect against oxidative stress, but the nature and/or regulation of these defenses in the vertebrate embryo is not well understood. The most abundant buffer of intracellular redox status is glutathione (GSH), a tripeptide of glutamate, cysteine, and glycine. Glutathione synthesis involves two ATP-dependent enzymatic steps (Fig. 1). In the first step, which is rate limiting, glutamate and cysteine are combined in a γ configuration, a reaction catalyzed by glutamate–cysteine ligase (Gcl). Gcl is composed of two subunits: a catalytic subunit, Gclc, and a modifier subunit, Gclm, that can lower the K_m of the catalytic subunit for glutamate and increase the K_i for GSH, thus controlling the rate of synthesis [31,32]. The expression of both *gclc* and *gclm* has been shown to respond to numerous factors including depleted GSH levels, ROS, reactive nitrogen species, cytokines, and various hormones [33–35]. The second step in GSH synthesis is performed by GSH synthase (Gss), which adds a glycine molecule to the fused γ-glutamate–cysteine (Fig. 1). Knockout of *gss* is embryonic lethal and *gss*-null mice fail to undergo gastrulation, demonstrating that GSH is essential for embryonic development [36]. Lu [37,38] provides a detailed review of GSH synthesis.

The GSH system sits at the nexus of antioxidant defense systems, xenobiotic metabolism, and epigenetic DNA methylation (Fig. 1). GSH serves as an antioxidant by scavenging ROS, which oxidize the cysteine moiety. Oxidation of GSH drives the formation of glutathione disulfide (GSSG), which can then be directly recycled to GSH through the enzyme glutathione disulfide reductase, a reaction requiring NADPH. GSH in the extracellular space can be recycled through the activity of γ-glutamyl transferase and the import of cysteine back into the cytoplasm to be reused in GSH synthesis (Fig. 1). The ratio of GSH to GSSG is often used as an indicator of intracellular redox status; however, with direct measurements of GSH and GSSG concentrations, one can use the Nernst equation to calculate the redox potential E_h [19,39–41]. It has been demonstrated previously that more negative E_h values (more highly reduced redox state) are associated with cell proliferation, and less negative E_h values (more highly oxidized redox state) are associated with differentiation and apoptosis [40–42]. GSH can also act as a cofactor for GSH-utilizing antioxidant enzymes, such as GSH peroxidase, glutaredoxin, and glutathione *S*-transferases (Fig. 1). In addition, GSH can modulate protein function, via S-thiolation or S-glutathionylation and the reversible formation of protein mixed disulfides (protein–SSG), as caused by shifts in intracellular redox status [43]. Another process with important implications for embryonic development is the interaction between GSH and epigenetic programming. The cysteine peptide in GSH is synthesized from the same pool of homocysteine that is used for the synthesis of *S*-adenosylmethionine, which serves as a cofactor for the methylation of DNA and histones [9]. Conditions that increase the synthesis of GSH—for example toxicant-induced GSH oxidation and/or depletion—can impair DNA methylation [44], suggesting that toxicants undergoing metabolism involving GSH could reduce the availability of homocysteine and thereby disrupt methylation.

Elucidating the many roles of GSH in ROS signaling, antioxidant defense, xenobiotic biotransformation, and epigenetics will require a better understanding of the ontogeny of GSH and its redox dynamics in the developing embryo. Surprisingly, little is known about GSH redox dynamics over the course of embryonic development in vertebrate systems. Progress has been hindered by the lack of a convenient model system and the difficulty in obtaining accurate measurements of reduced and oxidized glutathione.

The zebrafish embryo is a valuable vertebrate model system that is ideal for studying GSH redox dynamics during embryonic development. Zebrafish share many genetic, cellular, and physiological characteristics with mammals and are widely used as models in studies of vertebrate developmental biology and developmental

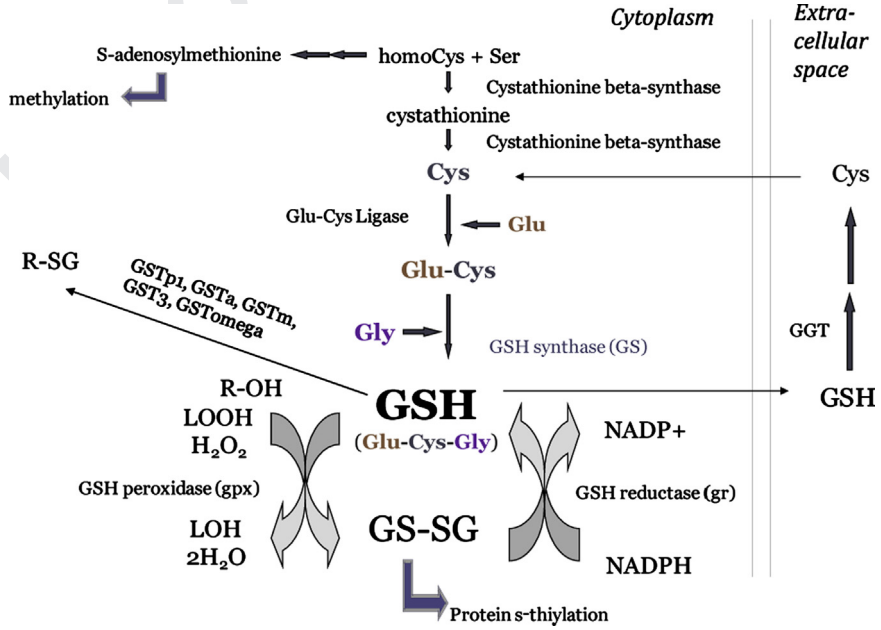

Fig. 1. Diagram of the glutathione redox system. Glutathione is a tripeptide of cysteine, glutamate, and glycine, which undergoes oxidation and forms a homodimer, GSSG. GSSG can participate in posttranslation modification of proteins by S-thiylation. GSSG can also be recycled back to reduced glutathione by GSH reductase in a reaction that utilizes NADPH. GSH can also be shuttled to the extracellular space and utilized, after which its cysteine component can be recycled by γ-glutamyl transferase (GGT). The synthesis of glutathione draws from cysteine pools synthesized from cystathionine, which is made from homocysteine. Glutathione thus draws from the same source of homocysteine that is necessary to maintain levels of S-adenosylmethionine needed for DNA methylation and epigenetic gene control, which is especially relevant during embryonic development.

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