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Original Research Communication

Immunohistochemical Localization of Thioredoxin and Glutaredoxin in Mouse Embryos and Fetuses

MIKIKO KOBAYASHI,[1] HAJIME NAKAMURA,[3] JUNJI YODOI,[3] and KOHEI SHIOTA[1,2]

ABSTRACT

Although oxygen is essential for promoting energy metabolism and for enhancing cell proliferation, early mouse embryos are very sensitive to high oxygen concentration. Because the tissue-specificity and sequential changes of the expression of antioxidative enzymes in rodent embryos have not been investigated systematically, we examined the ontogenesis of thioredoxin (TRX) and glutaredoxin (GRX) in mouse embryos and fetuses by using immunohistochemical methods. These compounds were found to be localized in most tissues examined, with some tissue specificity and temporal sequence. In many tissues, both TRX and GRX began to be expressed at embryonic day 11 (E11) or E13 and appeared to increase later in development, but the heart and great vessels of E8.5 embryos were already positive for their immunoreactivity. The stage at which the antioxidative enzymes begin to be expressed seems to coincide with the stage at which rodent embryos acquire the capacity of aerobic energy metabolism. Although TRX and GRX were co-localized in many tissues and showed similar sequential changes of expression, their expression patterns were different in the fetal cartilage, suggesting that they may play different roles in endochondral ossification. Their immunoreactivity was not homogeneous in the liver and the epithelium of uriniferous tubules, probably because their expression is associated with the proliferating and metabolic activities of the cell, as suggested by previous investigators. These results suggest that TRX and GRX play some tissue-specific roles in mammalian morphogenesis as well as general roles as antioxidant enzymes. Antiox. Redox Signal. 2, 653–663.

INTRODUCTION

RODENT EMBRYOS at the preimplantation and early postimplantation periods are extremely sensitive to high oxygen pressure. It has also been shown that the developmental block of preimplantation embryos at the two-cell stage *in vitro* (so-called "two-cell block") may be due to the inhibitory action of oxygen radicals and can be prevented by such antioxidant chemicals as thioredoxin (TRX) (Natsuyama *et al.*, 1992) and superoxide dismutase (SOD) (Noda *et al.*, 1991). Although early embryos are highly sensitive to oxygen, the oxygen requirement of rodent embryos increases rapidly during the later half of organogenesis (New and Coppola, 1970; New *et al.*, 1976). For some time after implantation, rodent embryos get oxygen and nutrients by diffusion through the chorion and yolk sac epithelium. Around embryonic day 9.5 (E9.5) in mice, the yolk sac placenta regresses and the allantoic placenta is

[1]Department of Anatomy and Developmental Biology and [2]Congenital Anomaly Research Center, Graduate School of Medicine, and [3]Department of Biological Response, Institute of Virus Research, Kyoto University, Kyoto 606-8501, Japan.

formed to take over an increasing share of respiratory and nutrient exchanges. Coincidentally with such transition of the placental structure, the energy metabolism in rodent embryos shifts from rather anaerobic to more aerobic processes, and the embryos become metabolically active and grow rapidly.

TRX is a small ubiquitous protein and functions as an antioxidative enzyme that catalyzes thiol-disulfide oxydoreductions (Holmgren, 1985). Human TRX was originally cloned as adult T-cell leukemia-derived factor (ADF) produced by human T-lymphotropic virus type-I (HTLV-I)-transformed T cells (Tagaya *et al.*, 1989; Yodoi and Tursz, 1991; Yodoi and Uchiyama, 1992). Glutaredoxin (GRX) is a glutathione-dependent hydrogen donor for ribonucleotide reductase and catalyzes glutathione-dependent disulfide oxidoreduction reactions (Holmgren, 1976, 1989). TRX and GRX belong to the TRX superfamily and have the same redox-active disulfide (Cys-X-Y-Cys) in common (Holmgren, 1989). They exist either in the oxidized form with a disulfide (-$(SH)_2$) or in the reduced form with a dithiol (S_2), and participate in the reversible oxidation-reduction reactions (Eklund *et al.*, 1984; Holmgren, 1985). They have been shown to be involved in a variety of biologically important reactions both in plants and animals (Holmgren, 1985; Nakamura *et al.*, 1997). Besides their originally described role as hydrogen donors, TRX and GRX are possibly involved in hormone secretion (Padilla *et al.*, 1992), cell proliferation (Gasdaska *et al.*, 1995), and cellular signaling (Rozell *et al.*, 1993). In addition, they have been suggested to interact with transcriptional proteins, such as AP-1 (Hirota *et al.*, 1997) and NF-κB (Okamoto *et al.*, 1992; Hayashi *et al.*, 1993; Hirota *et al.*, 1999). Moreover, TRX has chemotactic effects on monocytes, polymorphonuclear leucocytes, and T lymphocytes (Bertini *et al.*, 1999).

Previous immunohistochemical studies showed that TRX and GRX are expressed in cells and tissues that have a high activity of proliferation in animal and human fetuses (Rozell *et al.*, 1985, 1993; Fujii *et al.*, 1991). Fujii *et al.* (1991) examined immunohistochemically the TRX expression in human fetal tissues and demonstrated its localization in various tissues of human fetuses after 9 weeks of gestation. From this observation, they suggested that TRX might play some significant roles in the redox regulation in the human fetus. TRX has also been found to be localized in various tissues in adult rats (Rozell, 1985), and its distribution pattern was similar to that in human fetal tissues. GRX has also been shown to exist in varius tissues in the calf (Rozell *et al.*, 1993), whose distribution and occurrence were similar to those described for TRX in the rat. On the basis of these studies, Rozell *et al.* (1993) suggested that GRX might be involved in functions that are apart from the originally described role as a hydrogen donor.

Because the tissue specificity and sequential changes of TRX and GRX expression in rodent embryos have not been investigated systematically, we examined their localization and developmental changes in mouse embryos and fetuses by immunohistochemical methods. The results were compared with the corresponding data in the human and were discussed in relation to the critical period of organogenesis and metabolic changes occurring in developing mouse conceptuses.

MATERIALS AND METHODS

Animals

ICR strain mice were purchased from Japan SLC Co., Ltd. and reared in our laboratory. Virgin females were mated overnight with a male. They were checked for vaginal plugs next morning, and the noon of the day on which a vaginal plug was found was designated as E0.5.

Embryos and fetuses were collected by Caesarean section at days 8.5, 10.5, 11.5, 13.5, or 16.5 of gestation. They were fixed in 4% paraformaldehyde (wt/vol) in 0.1 *M* phosphate buffer, pH 7.2, overnight at 4°C. Then, they were dehydrated in graded concentrations of ethanol, embedded in paraffin, and sectioned at 10-μm thickness.

Immunohistochemistry

Deparaffinized sections were stained with rabbit polyclonal antibodies to mouse TRX

(Tomimoto *et al.*, 1993; Takagi *et al.*, 1998) and GRX (Takashima *et al.*, 1999) by ABC method. The sections were pretreated with 0.1% hydrogen peroxide for 30 min to remove endogenous peroxidase activity, and immersed in a blocking solution to reduce nonspecific background staining. Then, they were reacted with rabbit polyclonal antibodies to mouse TRX and GRX. The bound peroxidase was detected with diaminobenzidine (DAB), and the nuclei were counterstained with hematoxylin when appropriate.

RESULTS

Both TRX and GRX were detected in various tissues, and their distribution showed some tissue specificity and sequential changes. Usually

TABLE 1. LOCALIZATION OF THIOREDOXIN AND GLUTAREDOXIN IN TISSUES OF FETAL AND ADULT MICE

	Thioredoxin						*Glutaredoxin*					
Tissue	*8.5*	*10.5*	*11.5*	*13.5*	*16.5*	*Adult*	*8.5*	*10.5*	*11.5*	*13.5*	*16.5*	*Adult*
Neuroepithelium	+−	+−					+−	+−				
Surface ectoderm	−						−					
Mesenchyme	−	+	++	++	++		−	+	++	++	++	
Gut epithelium	−						−					
Cerebrum												
Cell body			+	+	+	+−			+	++	+	+−
Nerve fiber			+	++	+	+−			+	++	+	+−
Peripheral nerve												
Cell body			+	+	+	+−			+	+	+	+−
Nerve fiber			+	++	+	+−			+	++	+	+−
Spinal ganglion			+	+	+				+	+	+	
Epidermis		+−	+	+	+++			+−	+	+	+++	
Olfactory epithelium				++	++					++	++	
Hair follicle					++						++	
Eye												
Lens					++						++	
Retina				++	++						++	
Salivary gland					+						+	
Thyroid gland					+						++	
Heart												
Myocardium	++	++	++	++	++	+	++	++	++	++	++	+
Cartilage				++	++					++	++	
Muscle					++						++	
Kidney												
Glomerulus				+−	+−	+−				+−	+−	+−
Uriniferous tubules			+	++	+++	+++			+	+	+++	+++
Liver												
Hepatocyte			++	++	+	+−			++	++	++	+
Pancreas												
Acini					+						+	
Island					+						++	
Intestine												
Epithelium			+−	+	+	+				+−	+	+
Villous epithelium					+++	++					+++	++
Connective tissue			+	++	+	+			+	++	++	+
Lung												
Lung parenchyme				+	+	++				+	+	++
Bronchial epithelium				+	++	++				+	++	++
Decidua	+++	+++	+++	+++	+++		+++	+++	+++	+++	+++	
Syncytiotrophoblast		+++	+++	+++	+++			+++	+++	+++	+++	

Reactivity for the anti-mouse TRX and GRX antibodies: strongly positive (+++), moderately positive (++), weakly positive (+), faintly positive (+−), and negative (−).

their immunoreactivity was detected in the cytoplasm of the cell with the exception of such tissues as villous epithelium of the small intestine and pancreatic cells. TRX and GRX were co-localized in many tissues, and their immunoreactivity tended to increase as the developmental stage advanced. Both TRX and GRX were negative in most tissues of E8.5 mouse embryos except in the heart and great vessels, but their immunoreactivity began to be detectable in various tissues at E10.5 or E11.5 (Table 1). For example, the immunoreactivity of both TRX and GRX was negative in the surface ectoderm of E8.5 embryos, but it became slightly positive in the epidermis of E10.5 embryos and then increased by E11.5. Their immunoreactivity was intensely positive in the epidermis and the external root sheath of the body hair primordia in E16.5 fetuses (Fig. 1). The olfactory epithelium, which is a specialized ectodermal tissue, was intensely positive for TRX and GRX at E13.5. The retina and the lens, which are derived from the neuroepithelium and surface ectoderm, respectively, were similarly positive for TRX and GRX after E13.5. In the mesoderm-derived mesenchymal tissue, TRX and GRX immunoreactivity was not detected at E8.5, but was positive at E10.5 and then increased by E11.5. The visceral connective tissue and skeletal muscles were positive for the TRX and GRX immunostaining after E11.5. Both TRX and GRX were negative in the primitive gut epithelium (endoderm) of E8.5 embryos. TRX became faintly detectable in the intestinal epithelium at E11.5 and was distinctly positive at E13.5–16.5 (Fig. 2). GRX appeared in the intestinal epithelium later than TRX and was positive after E13.5 (Fig. 2). The villous epithelium of the fetal intestine showed intense immunoreactivity for both TRX and GRX at E16.5 in the nucleus (Fig. 2). Both TRX and GRX were intensely expressed, especially in the epithelial cells near the tip of intestinal villi. The connective tissue of the intestine was also positive for TRX and GRX, but their immunoreactivity was significantly less intense than that in the epithelial cells.

Interestingly, the heart and great vessels were positive for both TRX and GRX already at E8.5 (Fig. 3). The myocardium and the wall of great vessels showed strong immunoreactivity for both TRX and GRX from E11.5, and they remained positive until adult age. The epicardium and endocardium of the embryonic and fetal hearts were only slightly positive for both TRX and GRX, whose immunoreactivity was significantly less strong than that in the myocardium.

The neuroepithelium was only faintly positive for TRX and GRX at E8.5 and E10.5, but the differentiated neural tissue (both nerve cells and fibers) was clearly positive for them after E11.5. Their immunoreactivity appeared to peak at E13.5 and then became weaker by E16.5. Nucleated erythrocytes characteristic to the prenatal life were positive for TRX and GRX at E11.5, but the immunoreactivity became significantly weaker after E13.5.

Hepatocytes in the liver (Fig. 4) and acinous and islet cells in the pancreas (Fig. 5) showed positive immunoreactivity for TRX and GRX at fetal stages (E13.5 and E16.5). However, they showed heterogeneous localization in hepatic and pancreatic tissues. In the liver, TRX expression decreased in adult mice, whereas GRX was expressed continuously. In the pancreas, TRX and GRX were localized in the nucleus of

FIG. 1. Immunohistochemical localization of GRX in the surface ectoderm and skin. (**A**) Surface ectoderm and underlying mesenchyme of an E13.5 fetus. The GRX immunoreactivity is slightly recognizable in the surface ectoderm (*). (**B**) Skin of an E16.5 fetus. The GRX immunoreactivity is clearly positive in the epidermis (*). (**C**) Hair follicle of an E16.5 fetus. GRX is localized in the external root sheath of the primordial hair (arrowhead). (Bar = 10 μm.)

FIG. 2. Immunohistochemical localization of TRX (A–C) and GRX (D–F) in the small intestine. TRX is expressed slightly in the intestinal epithelial cells at E13.5 (**A**). At E16.5 (**B, E**) and in the adult (**C, F**), TRX and GRX are expressed heterogeneously in the villous epithelium. Their immunoreactivity is more intense at the distal part of intestinal villi. (Bar = 10 μm.)

FIG. 3. Immunohistochemical staining for TRX (A–D) and GRX (E–H) in embryonic, fetal, and adult mouse hearts. * indicates the myocardium. TRX and GRX are co-localized in the myocardium and their staining intensity is continuously positive during the prenatal life (**A–G**). TRX and GRX are expressed heterogeneously in muscle fibers of the adult heart (**D, H**). In the endocardium, both TRX and GRX are only slightly detectable. (Bar = 10 μm.) **A, E,** E8.5; **B, F,** E11.5; **C, G,** E16.5; **D, H,** adult.

FIG. 1.

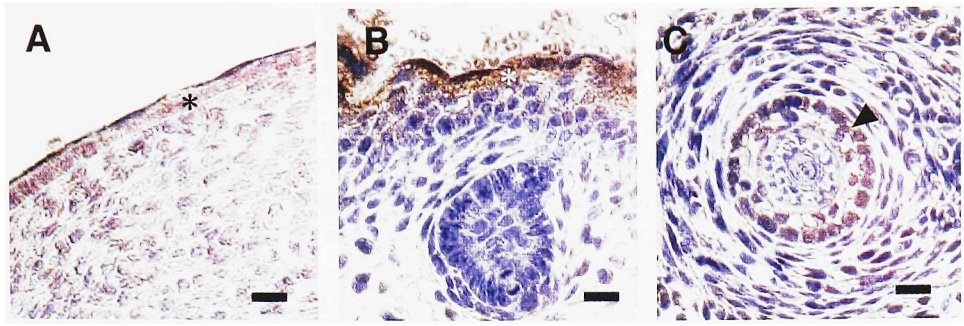


FIG. 2.

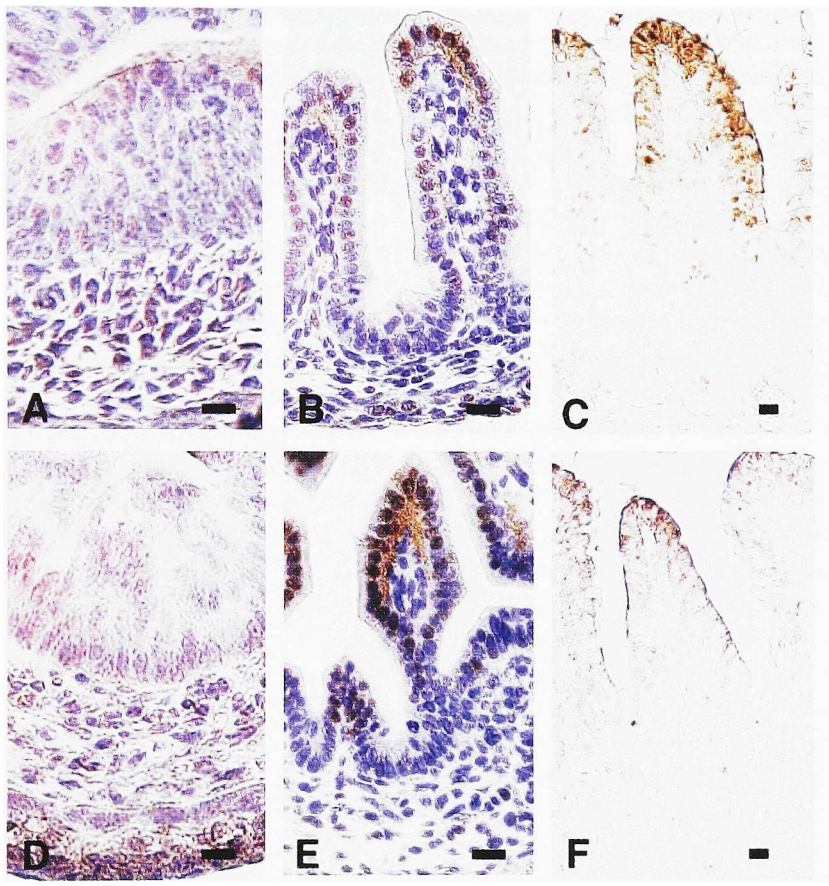
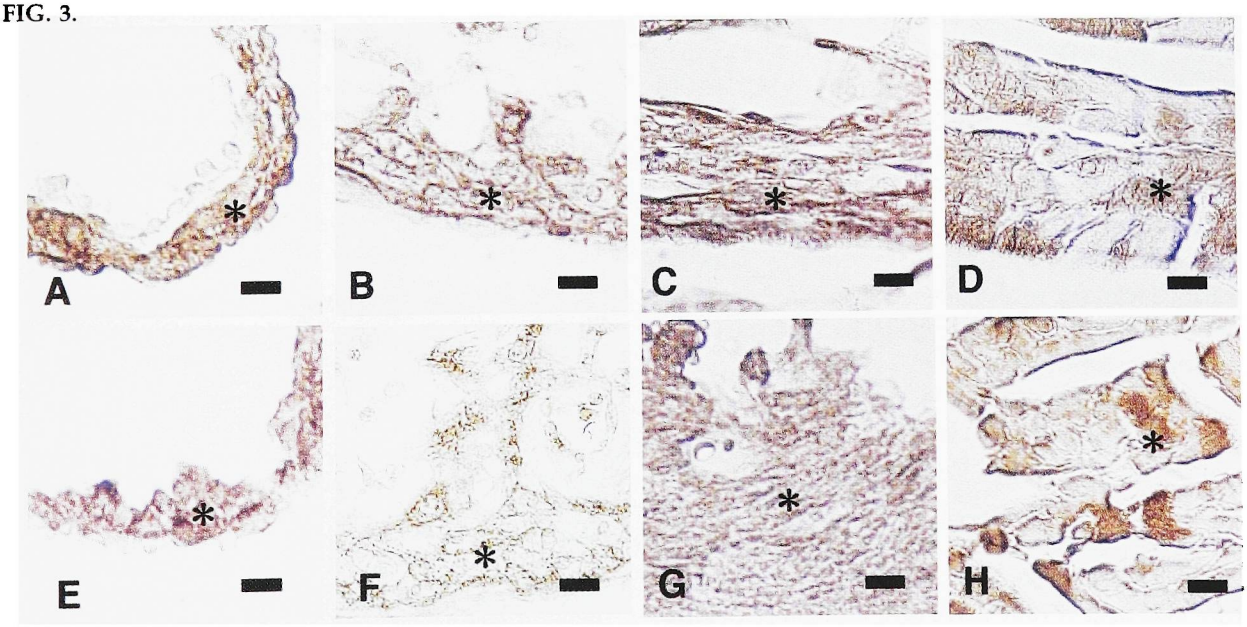


FIG. 3.



acinous and islet cells, and in islet cells they were also localized in the cytoplasm.

In the respiratory system, both the epithelial cells of the airway (future bronchial and alveolar epithelia) and the lung parenchyme showed positive immunoreactivity for TRX and GRX, which increased at later stages of development.

The epithelial cells of various exocrine and endocrine organs (*e.g.*, submandibular and thyroid glands) were positive for the immunostaining for TRX (data not shown) and GRX (Fig. 5) at E16.5. In the thyroid, the GRX immunoreactivity appeared more intense than that of TRX. A similar relationship between the two antioxidative enzymes was observed also in the liver and the connective tissue of the intestine.

In the kidney, the glomerular tissue was negative for both TRX (Fig. 6) and GRX (data not shown) both in fetal and adult mice. On the other hand, the epithelial cells of uriniferous tubules became positive for TRX and GRX from E11.5. Their immunoreactivity became more intense at later fetal stages (E13.5 and E16.5) and remained intensely positive in the adult kidney. The immunoreactivity for TRX and GRX was not homogeneous in the epithelial cells of uriniferous tubules.

Another interesting finding was observed in the cartilage (Fig. 7). TRX was expressed intensely in the cytoplasm of proliferating chondrocytes, whereas GRX was localized in hypertrophic chondrocytes that were in the process of apoptotic cell death. GRX was also expressed in proliferating chondrocytes, but it was much less intense than in hypertrophic chondrocytes.

The trophoblastic and decidual cells were both intensely positive for both TRX and GRX early in gestation (E8.5) and remained positive at later stages (data not shown). The immunoreactivity in these extraembryonic tissues was significantly stronger than in embryonic proper tissues.

DISCUSSION

In the present study, TRX and GRX were found to be present in most tissues of mouse embryos and fetuses during and after organogenesis, and they showed some spatial heterogeneity and temporal sequences. They tended to be co-localized in many tissues, although their intensity and initiation of expression were not exactly identical. In many tissues, both TRX and GRX began to be expressed at E10.5 or E11.5 and their immunoreactivity increased between E11.5 and E13.5 and often peaked at E16.5. Such temporal sequence of their expression seems to coincide with the progression of organogenesis and histogenesis. They appeared to be expressed in growing and metabolically active tissues and in proliferating cells, as has been suggested by Rozell *et al.* (1985, 1993) and Fujii *et al.* (1991).

Although TRX and GRX were generally localized in the cytoplasm, they were expressed also in the nucleus in some tissues such as villous epithelium of the small intestine and pancreatic cells. Hirota *et al.* (1997) showed that TRX localization migrated from the cytoplasm to the nucleus in HeLa cells treated with phorbol acetate. It may be that the localization of TRX and GRX in the cell may reflect the functional state of the cell or its phase in the cell cycle.

FIG. 4. Immunohistochemical staining for TRX (A–C) and GRX (D–F) in the liver. TRX and GRX are expressed in hepatocytes but their immunoreactivity is heterogeneous. TRX expression in the liver decreases in E16.5 fetuses (**C**) and adult mice. On the other hand, GRX expression increases with development (**D–F**). (Bar = 10 μm.) **A, D,** E11.5; **B, E,** E13.5; **C, F,** E16.5.

FIG. 5. Immunohistochemical localization of GRX in the pancreas (A) and thyroid gland (B) at E16.5 (**A**) Pancreas. GRX is localized in islet cells (*) and less intensively in acinar epithelial cells. Its immunoreactivity is heterogeneous in the epithelial cells of acini. (**B**) Thyroid gland. GRX is expressed heterogeneously in glanular cells of the thyroid. (Bar = 10 μm.)

FIG. 6. Immunohistochemical localization of TRX (A, B) in the kidney. TRX is heterogeneously expressed in the nuclei of the epithelial cells of uriniferous tubules, but is negative in glomeruli (*) (Bar = 10 μm.) **A,** E16.5; **B,** adult.

FIG. 7. Immunohistochemical localization of GRX (A) and TRX (B) in endochondral ossification in the limb of E16.5 mice. (**A**) Hypertrophic chondrocytes that are in the process of apoptosis are positive for GRX immunoreactivity. Chondrocytes at the proliferating zone (*) are only faintly positive for GRX. (**B**) TRX is expressed in both proliferating and hypertrophic chondrocytes. (Bar = 10 μm.).

FIG. 4.

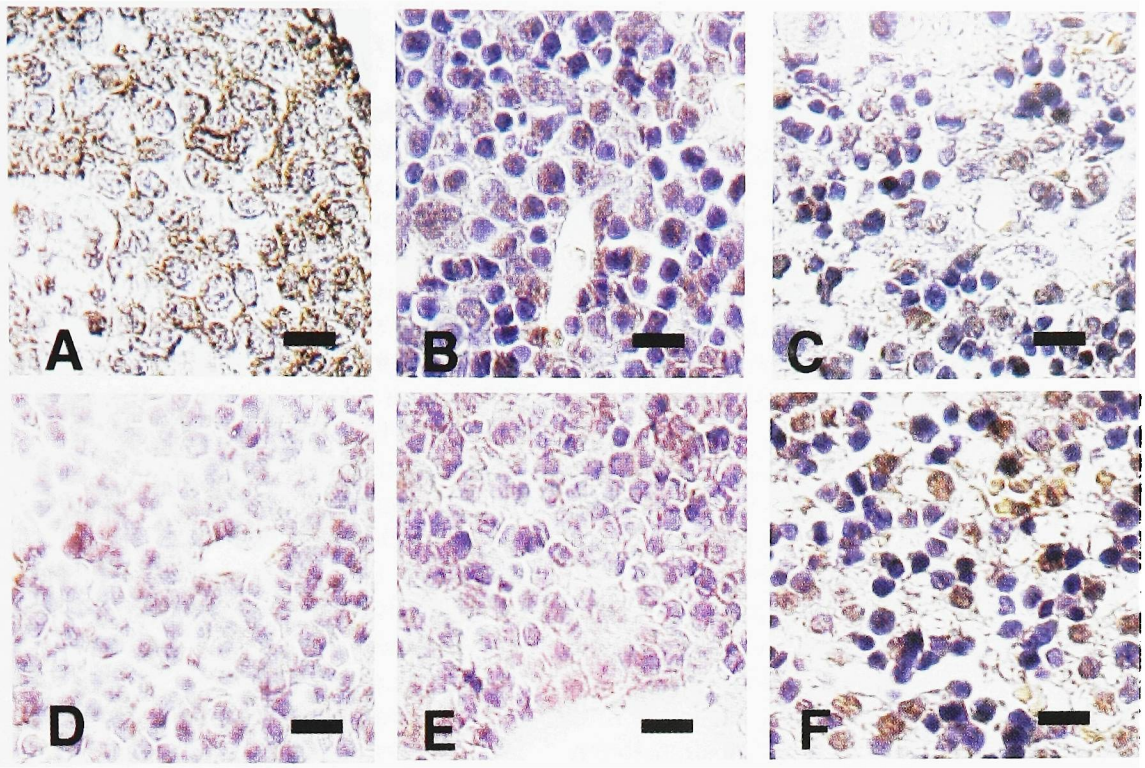


FIG. 5.

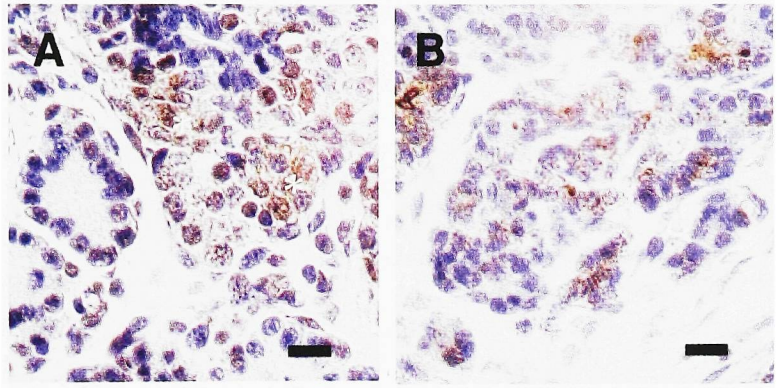


FIG. 6.

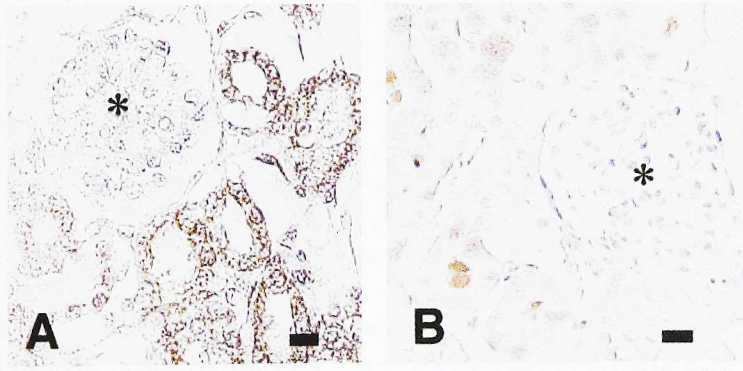
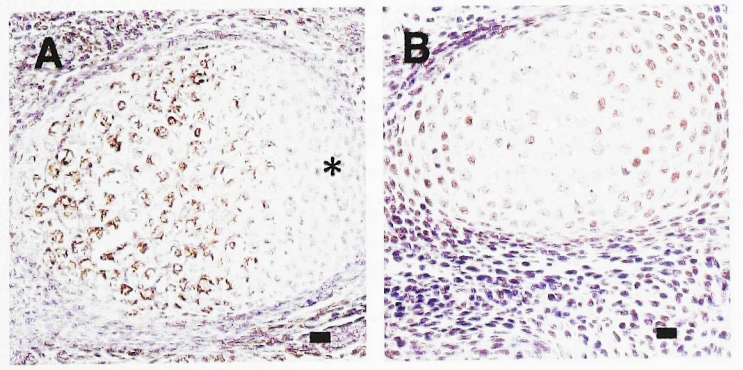


FIG. 7.



In E8.5 neurulating embryos, TRX and GRX were negative except in the neuroepithelium and the myocardium. Their immunoreactivity in the former tissue was only slightly recognizable, but the myocardium was already intensely positive for both enzymes as early as E8.5. The myocardium was continuously positive for TRX and GRX during the prenatal life, and their immunoreactivity became less intense in the myocardial tissue of adult mice. This finding was interesting because the heart and great vessels are among the major organs that are formed first in the embryo and play essential roles in early development. On the other hand, Rozell *et al.* (1985) examined the tissues of the adult rat immunohistochemically and found that the cardiac muscle was negative for TRX, which was not consistent with our finding in adult mice. The reason for such inconsistency between the mouse and rat studies is not clear but it may be due to some genetically determined species difference or to a difference in the antibodies used in the two studies.

The cerebrum was found to become intensely positive for TRX and GRX at E11.5 and then their immunoreactivity declined by E16.5. In the mouse embryo, the production and migration of neuronal cells start around E11. They are actively produced at E11–14 and their production ceases by E17 (Kameyama and Hoshino, 1986). Therefore, the sequential changes of TRX and GRX expression seem to reflect the temporal sequence of neuron production in the mouse brain. With this regard, it is interesting to note the finding by Fujii *et al.* (1991) that TRX was negative in the neurons in the central nervous system (CNS) of human fetuses after 9 weeks of gestation when neuronal proliferation is not active or has ceased.

In the digestive tract, one of the remarkable findings was that TRX and GRX were intensely positive in the apical villous epithelium of the small intestine, whereas their immunoreactivity was significantly weaker in the proximal part of the villous epithelium and in the connective tissue of the gut wall. Although the intestinal epithelium of the mouse fetus is not actually functioning yet, the differential localization of TRX and GRX may reflect some different states of differentiation in the apical and proximal parts of the villous epithelium. In the human fetus, the epithelial lining of the esophagus, stomach, and intestine show moderate to strong immunoreactivity for TRX, which may be dependent on their degree of differentiation and functional state (Fujii *et al.*, 1991).

Both TRX and GRX were intensely positive in the liver of E11.5 and E13.5 fetuses, and TRX became less intense in the hepatic tissue of E16.5 fetuses and adult mice. The enzymes were localized in hepatocytes, but their intensity was not homogeneous among hepatocytes. At this stage of fetal development, hepatocytes are actively proliferating and the fetal liver has a hematopoietic function. In the human fetus also, TRX is expressed more intensely than in the adult liver (Fujii *et al.*, 1991). Nakamura *et al.* (1992) have shown that TRX was expressed in the adult human liver and that its immunoreactivity significantly increased in human hepatoma cells. On the other hand, GRX was continuously expressed in the liver of fetal and adult mice and was detected both in the nuclei and cytoplasm of the hepatocyte. GRX may participate in active metabolism in the liver depending on the GSH redox regulating system (Holmgren, 1976, 1989).

In the pancreas of E16.5 fetuses, TRX and GRX were expressed heterogeneously in the nuclei of acinar cells and in the cytoplasm of islet cells. In the human fetus and adult rat, TRX was detected in acinar and islet cells (Rozell *et al.*, 1985; Fujii *et al.*, 1991). TRX was present also in the glandular cells of the thyroid and submandibular glands. These findings support the previous reports indicating that TRX is associated with active secretory function (Padilla *et al.*, 1992) and has chemotactic effects (Bertini *et al.*, 1999).

In the kidney, TRX and GRX were detected heterogeneously in the lining cells of uriniferous tubules, but were negative in glomeruli. Why the expression of these enzymes is not homogeneous in the uriniferous epithelium is not known, but the sensitivity to oxidative stress may differ among tubules depending on their functional state. Toyokuni and his colleagues examined the expression of TRX in the kidney of a mouse model of free radical-

associated renal carcinogenesis and found that TRX is expressed heterogeneously in uriniferous tubules (Tanaka *et al.*, 1997; Toyokuni *et al.*, 1999). It is possible that TRX and GRX may regulate the redox state in the uriniferous epithelial cells whose functional state may not be identical among nephrons, depending on their secretory and reabsorbing activity.

TRX and GRX were found to be expressed differently during endochondral ossification. TRX was expressed in chondrocytes at the proliferating zone, especially in their nuclei. On the other hand, GRX was only slightly expressed in proliferating chondrocytes but was intensely detectable in the nuclei and cytoplasm of hypertrophic chondrocytes that undergo apoptotic death to be replaced by osteogenic tissue. The reason is not clear why TRX and GRX are expressed differently in the fetal cartilage, but they may be involved in different metabolic processes in chondrocytes during endochondral ossification.

In the extraembryonic tissue, TRX and GRX were intensely expressed in decidual and trophoblastic tissues early in development (E8.5). Kobayashi *et al.* (1995) examined human placental tissues and found that human TRX (ADF) was abundantly present in human decidual and trophoblastic cells, which is consistent with our present result in the mouse. It is likely that TRX and GRX take part in protecting the implanted conceptus from the cytotoxic effects of oxygen radicals generated by the rapid blood flow in the placenta. It is interesting to note a previous finding that mouse embryos lacking the TRX gene failed to implant or died shortly after implantation (Matsui *et al.*, 1996). Therefore, TRX may be essential for early development and differentiation of rodent embryos.

TRX and GRX are expressed in various tissues of mouse and human embryos, and their expression patterns show some spatial heterogeneity and temporal sequence. In addition to their primary role in the oxidoreduction process, they may be involved in cell proliferation and differentiation in various tissues, as suggested by previous studies, although their roles can differ in different tissues. Experimental studies are in progress in our laboratories to elucidate the roles of these enzymes in ontogenesis and tissue differentiation.

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ABBREVIATIONS

ADF, Adult T-cell leukemia-derived factor (human thioredoxin); AP-1, activator protein-1; CNS, central nervous system; DAB, diaminobenzidine; GRX, glutaredoxin; HTLV-I, human T lymphotropic virus type-I; NF-κB, nuclear factor-κB; SOD, superoxide dismutase; TRX, thioredoxin.

REFERENCES

BERTINI, R., HOWARD, O.M., DONG, H.F., OPPENHEIM, J.J., BIZZARRI, C., SERGI, R., CASELLI, G., PAGLIEI, S., ROMINES, B., WILSHIRE, J.A., MENGOZZI, M., NAKAMURA H., YODOI, J., PEKKARI, K., GURUNATH, R., HOLMGREN, A., HERZENBERG, L.A., HERZENBERG, L.A., and GHEZZI, P. (1999). Thioredoxin, a redox enzyme released in infection and inflammation, is a unique chemoattractant for neutrophils, monocytes, and T cells. J. Exp. Med. **189,** 1783–1789.

EKLUND, H., CAMBILLAU, C., SJOBERG, B.M., HOLMGREN, A., JORNVALL, H., HOOG, J.O., and BRANDEN, C.I. (1984). Conformational and functional similarities between glutaredoxin and thioredoxins. EMBO J. **3,** 1443–1449.

FUJII, S., NANBU, Y., KONISHI, I., MORI, T., MASUTANI, H., and YODOI, J. (1991). Immunohistochemical localization of adult T-cell leukaemia-derived factor, a human thioredoxin homologue, in human fetal tissues. Virchow's Arch. A Pathol. Anat. Histopathol. **419,** 317–326.

GASDASKA, J.R., BERGGREN, M., and POWIS, G. (1995). Cell growth stimulation by the redox protein thioredoxin occurs by a novel helper mechanism. Cell Growth Differ. **6,** 1643–1650.

HAYASHI, T., UENO, Y., and OKAMOTO, T. (1993). Oxidoreductive regulation of nuclear factor kappa B. Involvement of a cellular reducing catalyst thioredoxin. J. Biol. Chem. **268,** 11380–11388.

HIROTA, K., MATSUI, M., IWATA, S., NISHIYAMA, A., MORI, K., and YODOI, J. (1997). AP-1 transcriptional activity is regulated by a direct association between thioredoxin and Ref-1. Proc. Natl. Acad. Sci. USA **94,** 3633–3638.

HIROTA, K., MURATA, M., SACHI, Y., NAKAMURA, H., TAKEUCHI, J., MORI, K., and YODOI, J. (1999). Distinct roles of thioredoxin in the cytoplasm and in the nucleus. A two-step mechanism of redox regulation of transcription factor NF-kappaB. J. Biol. Chem. **274,** 27891–27897.

HOLMGREN, A. (1976). Hydrogen donor system for Escherichia coli ribonucleoside-diphosphate reductase dependent upon glutathione. Proc. Natl. Acad. Sci. USA **73,** 2275–2279.

HOLMGREN, A. (1985). Thioredoxin. Annu. Rev. Biochem. **54,** 237–271.

HOLMGREN, A. (1989). Thioredoxin and glutaredoxin systems. J. Biol. Chem. **264,** 13963–13966.

KAMEYAMA, K., and HOSHINO, K. (1986). Radiation risks to the developing nervous system. In: *Sensitive Phase of CNS Development*. H. Kriegel, ed. (Gustav Fischer, Stuttgart) pp. 75–92.

KOBAYASHI, F., SAGAWA, N., NANBU, Y., KITAOKA, Y., MORI, T., FUJII, S., NAKAMURA, H., MASUTANI, H., and YODOI, J. (1995). Biochemical and topological analysis of adult T-cell leukaemia-derived factor, homologous to thioredoxin, in the pregnant human uterus. Hum. Reprod. **10,** 1603–1608.

MATSUI, M., OSHIMA, M., OSHIMA, H., TAKAKU, K., MARUYAMA, T., YODOI, J., and TAKETO, M.M. (1996). Early embryonic lethality caused by targeted disruption of the mouse thioredoxin gene. Dev. Biol. **178,** 179–185.

NAKAMURA, H., MASUTANI, H., TAGAYA, Y., YAMAUCHI, A., INAMOTO, T., NANBU, Y., FUJII, S., OZAWA, K., and YODOI, J. (1992). Expression and growth-promoting effect of adult T-cell leukemia-derived factor. A human thioredoxin homologue in hepatocellular carcinoma. Cancer **69,** 2091–2097.

NAKAMURA, H., NAKAMURA, K., and YODOI, J. (1997). Redox regulation of cellular activation. Annu. Rev. Immunol. **15,** 351–369.

NATSUYAMA, S., NODA, Y., NARIMOTO, K., UMAOKA, Y., and MORI, T. (1992). Release of two-cell block by reduction of protein disulfide with thioredoxin from Escherichia coli in mice. J. Reprod. Fertil. **95,** 649–656.

NEW, D.A., and COPPOLA, P.T. (1970). Effects of different oxygen concentrations on the development of rat embryos in culture. J. Reprod. Fertil. **21,** 109–118.

NEW, D.A., COPPOLA, P.T., and COCKROFT, D.L. (1976). Improved development of head-fold rat embryos in culture resulting from low oxygen and modifications of the culture serum. J. Reprod. Fertil. **48,** 219–222.

NODA, Y., MATSUMOTO, H., UMAOKA, Y., TATSUMI, K., KISHI, J., and MORI, T. (1991). Involvement of superoxide radicals in the mouse two-cell bock. Mol. Reprod. Dev. **28,** 356–360.

OKAMOTO, T., OGIWARA, H., HAYASHI, T., MITSUI, A., KAWABE, T., and YODOI, J. (1992). Human thioredoxin/adult T cell leukemia-derived factor activates the enhancer binding protein of human immunodeficiency virus type 1 by thiol redox control mechanism. Int. Immunol. **4,** 811–819.

PADILLA, C.A., MARTINEZ GALISTEO, E., LOPEZ BAREA, J., HOLMGREN, A., and BARCENA, J.A. (1992). Immunolocalization of thioredoxin and glutaredoxin in mammalian hypophysis. Mol. Cell. Endocrinol. **85,** 1–12.

ROZELL, B., HANSSON, H.A., LUTHMAN, M., and HOLMGREN, A. (1985). Immunohistochemical localization of thioredoxin and thioredoxin reductase in adult rats. Eur. J. Cell Biol. **38,** 79–86.

ROZELL, B., BARCENA, J.A., MARTINEZ GALISTEO, E., PADILLA, C.A., and HOLMGREN, A. (1993). Immunochemical characterization and tissue distribution of glutaredoxin (thioltransferase) from calf. Eur. J. Cell Biol. **62,** 314–323.

TAGAYA, Y., MAEDA, Y., MITSUI, A., KONDO, N., MATSUI, H., HAMURO, J., BROWN, N., ARAI, K., YOKOTA, T., WAKASUGI, H., and YODOI, J. (1989). ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. EMBO J. **8,** 757–764.

TAKAGI, Y., TOKIME, T., NOZAKI, K., GON, Y., KIKUCHI, H., and YODOI, J. (1998). Redox control of neuronal damage during brain ischemia after middle cerebral artery occlusion in the rat: immunohistochemical and hybridization studies of thioredoxin. J. Cereb. Blood Flow Metab. **18,** 206–214.

TAKASHIMA, Y., HIROTA, K., NAKAMURA, H., NAKAMURA, T., AKIYAMA, K., CHENG, F.S., MAEDA, M., and YODOI, J. (1999). Differential expression of glutaredoxin and thioredoxin during monocytic differentiation. Immunol. Lett. **68,** 397–401.

TANAKA, T., NISHIYAMA, Y., OKADA, K., HIROTA, K., MATSUI, M., YODOI, J., HIAI, H., and TOYOKUNI, S. (1997). Induction and nuclear translocation of thioredoxin by oxidative damage in the mouse kidney: independence of tubular necrosis and sulfhydryl depletion. Lab. Invest. **77,** 145–155.

TOMIMOTO, H., AKIGUCHI, I., WAKITA, H., KIMURA, J., HORI, K., and YOKOI, J. (1993). Astroglial expression of ATL-derived factor, a human thioredoxin homologue, in the gerbil brain after transient global ischemia. Brain Res. **625,** 1–8.

TOYOKUNI, S., IWASA, Y., KONDO, S., TANAKA, T., OCHI, H., and HIAI, H. (1999). Intranuclear distribution of 8-hydroxy-2′-deoxyguanosine. An immunocytochemical study. J. Histochem. Cytochem. **47,** 833–836.

YODOI, J., and TURSZ, T. (1991). ADF, a growth-promoting factor derived from adult T cell leukemia and

homologous to thioredoxin: involvement in lymphocyte immortalization by HTLV-I and EBV. Adv. Cancer Res. **57,** 381–411.

YODOI, J., and UCHIYAMA, T. (1992). Diseases associated with HTLV-I: virus, IL-2 receptor dysregulation and redox regulation. Immunol. Today **13,** 405–411.

YODOI, J., TAGAYA, Y., MASUTANI, H., MAEDA, Y., and KAWABE, T. (1988). IL-2 receptor and Fc epsilon R2 gene activation in lymphocyte transformation: possible roles of ATL-derived factor. Princess Takamatsu Symp. **19,** 73–86.

Address reprint requests to:
Dr. Kohei Shiota
Department of Anatomy and Developmental Biology
Kyoto University
Kyoto 606-8501, Japan

E-mail: kshiota@med.kyoto-u.ac.jp

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2. José Rodrigo Godoy, Maria Funke, Waltraud Ackermann, Petra Haunhorst, Sabrina Oesteritz, Francisco Capani, Hans-Peter Elsässer, Christopher Horst Lillig. 2011. Redox atlas of the mouse. *Biochimica et Biophysica Acta (BBA) - General Subjects* **1810**:1, 2-92. [CrossRef]
3. Y. Kamimoto, T. Sugiyama, T. Kihira, L. Zhang, N. Murabayashi, T. Umekawa, K. Nagao, N. Ma, N. Toyoda, J. Yodoi, N. Sagawa. 2010. Transgenic mice overproducing human thioredoxin-1, an antioxidative and anti-apoptotic protein, prevents diabetic embryopathy. *Diabetologia* **53**:9, 2046-2055. [CrossRef]
4. Kelly F. Portes, Cecília M. Ikegami, Joselito Getz, Ana P. Martins, Lucia Noronha, Luciana F. Zischler, Giseli Klassen, Anamaria A. Camargo, Silvio M. Zanata, Estela Bevilacqua, Lia S. Nakao. 2008. Tissue distribution of quiescin Q6/sulfhydryl oxidase (QSOX) in developing mouse. *Journal of Molecular Histology* **39**:2, 217-225. [CrossRef]
5. L. Zhao, Y.-H. Chen, H. Wang, Y.-L. Ji, H. Ning, S.-F. Wang, C. Zhang, J.-W. Lu, Z.-H. Duan, D.-X. Xu. 2007. Reactive Oxygen Species Contribute to Lipopolysaccharide-Induced Teratogenesis in Mice. *Toxicological Sciences* **103**:1, 149-157. [CrossRef]
6. E RADDATZ, S GARDIER, A SARRE. 2006. Physiopathology of the embryonic heart (with special emphasis on hypoxia and reoxygenation). *Annales de Cardiologie et d'Angéiologie* **55**:2, 79-89. [CrossRef]
7. Kumuda C. Das . 2004. Thioredoxin System in Premature and Newborn Biology. *Antioxidants & Redox Signaling* **6**:1, 177-184. [Abstract] [Full Text PDF] [Full Text PDF with Links]