

Developmental Expression of Glutathione Peroxidase, Catalase, and Manganese Superoxide Dismutase mRNAs During Spermatogenesis in the Mouse

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ABSTRACT: We have examined in mouse testis the steady-state levels of mRNAs encoding glutathione peroxidase (GSHPx), catalase (CAT), and superoxide dismutase 2 (SOD-2), three enzymes essential for the antioxidant protection of cells. In RNA preparations derived from prepuberal and adult testes and from isolated populations of meiotic and post-meiotic germ cells, one major GSHPx mRNA of about 0.8 kilobases (kb) and one major CAT mRNA of about 2.4 kb were detected. Three SOD-2 mRNAs of about 2.2, 1.2, and 1.0 kb were found in testis. In contrast to GSHPx and CAT, the mRNA levels of SOD-2 were higher in testis than in liver. SOD-2

mRNA levels are developmentally and translationally regulated with maximal levels of expression in early post-meiotic germ cells, whereas the levels of GSHPx and CAT mRNAs are relatively constant in both prepuberal and adult testes. These data suggest that translational regulation plays a more prominent role for SOD-2 expression than for GSHPx or CAT expression in the mammalian testis.

Key words: Antioxidant enzymes, gene expression, testis, translational regulation.

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The damage produced by forms of reactive oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^\bullet) is believed to be a major contributor to aging and to degenerative processes including cancer, heart disease, and brain dysfunction (Harman, 1981, 1992; Stadtman, 1992; see Ames et al, 1993 for review). Even under normal physiological conditions, endogenous oxygen radicals generated as by-products of cellular metabolism result in extensive oxidative damage to molecules, leading to DNA damage, protein inactivation, and cell membrane instability (Fridovich, 1978; Peskin and Shlyahova, 1986; Davies, 1987; Wagner et al, 1992).

An important enzyme pathway has evolved to protect eukaryotic cells against oxidative damage. Superoxide dismutases (SOD) scavenge O_2^- by converting it to H_2O_2 (see Fridovich, 1978 for review), which in turn is broken down to H_2O in the cytoplasm by glutathione peroxidase (GSHPx) (Chance et al, 1979) and in peroxisomes by catalase (CAT) (Aebi, 1984). GSHPx also reduces cellular organic peroxides. There are two major intracellular types of SOD, copper-zinc SOD (SOD-1) and manganese SOD (SOD-2). SOD-1 is primarily a cytosolic enzyme that functions in both the cytoplasm and nucleus (Crapo et al,

1992), whereas SOD-2 is localized to mitochondria (Barra et al, 1984). These enzymes function together to provide an important protection mechanism for tissues against oxidative attack.

Oxidative lesions in germline DNA are of special concern because they are likely to increase the incidence of genetic diseases and cancer in progeny (National Research Council, 1990). Certain genetic abnormalities, such as those induced by mutations in the retinoblastoma gene (Dryja et al, 1989) arise from germline mutations and display a high frequency of paternal origin (Vogel and Rathenberg, 1975; Mastuda et al, 1989; Crow, 1993). In addition, oxidative damage in human spermatozoa has been associated with loss of motility and decreased fusion capability, leading to loss of fertility (Aitken and Clarkson, 1987; Alvarez et al, 1987; Aitken et al, 1989, 1991). Antioxidant enzymes play a crucial role in protecting male germ cells from oxidative damage (Holland et al, 1982; Alvarez and Storey, 1989, 1992; Bauche et al, 1994). Bauche et al (1994) demonstrated that pachytene spermatocytes, round spermatids, and spermatozoa contain 38–56% more SOD activity than testicular somatic cells. SOD-1, GSHPx, and CAT proteins have been reported to be present in mammalian sperm and appear necessary for sperm motility (Alvarez and Storey, 1989, 1992; Jeulin et al, 1989). Furthermore, it has been shown that the seminal plasma of various mammals, including humans, contains significant amounts of SOD-1 and CAT activity (Menella and Jones, 1980; Jeulin et al, 1989; Nanogaki et al, 1992), suggesting that they also play important physiological roles in the ejaculate.

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Although the presence of antioxidant enzymes in many of the cells of the testis is established, the mechanisms regulating their expression in male germ cells have not been studied in detail. We have previously examined the regulation of SOD-1 gene expression during spermatogenesis in the mouse and identified multiple SOD-1 mRNAs, including a testis-specific SOD-1 transcript that is transcribed in post-meiotic cells and is translationally controlled (Gu et al, 1995a). Here we use specific cDNA probes to monitor the gene expression of GSHPx, CAT, and SOD-2 in prepuberal and adult testes and in purified populations of germ cells. In addition, we have also examined the translational status of GSHPx, CAT, and SOD-2 mRNAs in adult mouse testes.

Materials and Methods

Isolation of Enriched Populations of Male Germ Cells

CD-1 male mice were purchased from Charles River Laboratories (Wilmington, Massachusetts). Enriched populations of pachytene spermatocytes, round spermatids, and elongating spermatids were prepared by the unity gravity sedimentation procedure, as previously described (Gu et al, 1995b). The purity of the separated germ cells was monitored by phase-contrast microscopy and by northern blot hybridization with a mouse cDNA probe encoding protamine 2 (Kleene et al, 1984).

RNA Isolation and Northern Blot Hybridization

Total RNA was isolated from the liver, brain, and testes of adult mice, from the testes of prepuberal mice at 6, 12, 17, 22, and 25 days after birth, and from isolated germ cell populations, as described by Alcivar et al (1989). Northern blot hybridizations were performed as previously described (Gu et al, 1995b).

Three gene-specific cDNA probes were used in this study. A cDNA probe of about 0.6 kilobases (kb) that encodes mouse GSHPx was generated from a mouse testis cDNA library by polymerase chain reaction (PCR) amplification. The second probe was a 0.5-kb cDNA encoding the C-terminus of the mouse CAT protein generated by PCR from a full-length CAT cDNA clone, kindly provided by Dr. R. N. Ott (Division of Molecular Genetics, Western Science Center, London, Ontario, Canada). The third probe was a 0.83-kb human SOD-2 cDNA purchased from the American Type Culture Collection (cat. no. 59946; Rockville, Maryland).

Fractionation of Post-mitochondrial Extracts

The fractionation of post-mitochondrial supernatants of adult testis by sucrose gradient centrifugation was performed as previously described (Kleene et al, 1984; Alcivar et al, 1989). RNA was isolated from the fractionated polysomal gradients and northern blot hybridizations were performed as previously described (Gu et al, 1995a).

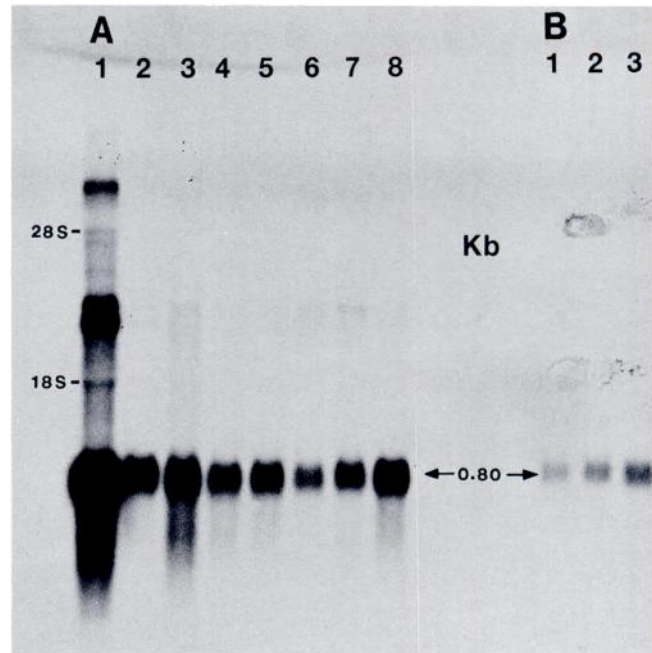


FIG. 1. Northern blot analysis of GSHPx mRNAs in total RNA from mouse liver, brain, prepuberal testes, and adult testes. **(A)**, Aliquots of RNA (10 μ g) from adult mouse liver (lane 1), brain (lane 2), and testes from 6-day-old (lane 3), 12-day-old (lane 4), 17-day-old (lane 5), 22-day-old (lane 6), 25-day-old (lane 7), and adult (lane 8) mice were electrophoresed in 1% agarose gels and transferred to nylon membranes. The membranes were hybridized with a 32 P-labeled cDNA encoding mouse GSHPx. The arrow indicates the position of the predominant GSHPx mRNA. **(B)**, Aliquots of RNA (10 μ g) from pachytene spermatocytes (lane 1), round spermatids (lane 2), and elongating spermatids (lane 3) were analyzed as in A. To ensure that equal amounts of RNA were loaded in each lane and equal amounts were transferred to the nylon membranes, RNAs were monitored in the gel before and after RNA transfer.

Results

Developmental Expression of Glutathione Peroxidase (GSHPx) mRNA in the Mouse Testis

Hybridizing total RNA from mouse liver, brain, and sexually immature and mature testes with a mouse GSHPx cDNA, we detected one abundant GSHPx transcript of about 0.8 kb (Fig. 1A, lanes 1–8). When equal amounts of RNA were analyzed, higher levels of GSHPx were observed in the liver than testis or brain (Fig. 1A, compare lane 1 to lanes 2 and 3). Moreover, additional GSHPx transcripts with slower electrophoretic mobilities were also seen in the liver (Fig. 1A, lane 1).

To better define the relative levels of GSHPx mRNAs in germ cell populations in the testis, northern blot hybridizations were performed with RNAs isolated from meiotic and post-meiotic male germ cells (Fig. 1B). We detected a gradually increasing amount of the 0.8-kb GSHPx mRNA as pachytene spermatocytes (Fig. 1B, lane 1) differentiated to elongating spermatids (Fig. 1B, lane

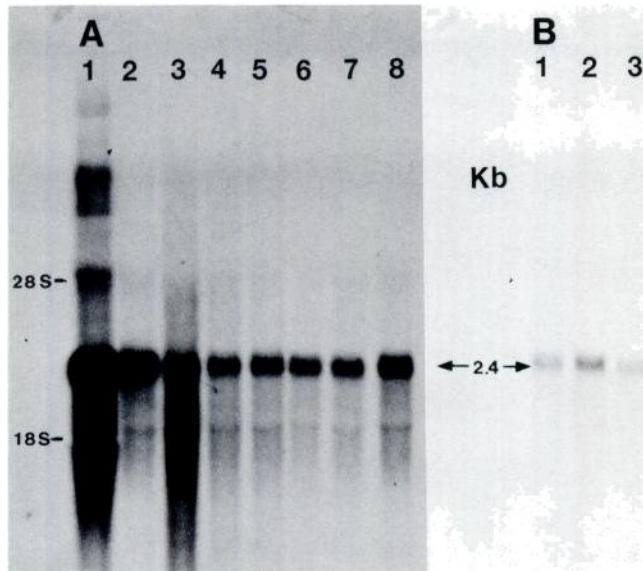


FIG. 2. Northern blot analysis of CAT mRNAs in total RNA from mouse liver, brain, prepubertal testes, and adult testes. **(A)**, RNA aliquots (10 μ g) from adult mouse liver (lane 1), brain (lane 2), and testes from 6-day-old (lane 3), 12-day-old (lane 4), 17-day-old (lane 5), 22-day-old (lane 6), 25-day-old (lane 7), and adult (lane 8) mice were electrophoresed in 1% agarose gels and transferred to nylon membranes. The membranes were hybridized with a 32 P-labeled cDNA encoding mouse CAT. The arrow indicates the position of the major CAT mRNA. **(B)**, Aliquots of RNA (10 μ g) from pachytene spermatocytes (lane 1), round spermatids (lane 2), and elongating spermatids (lane 3) were analyzed as in A.

3). When equal amounts of RNA were analyzed, GSHPx mRNA levels appeared to be lower in meiotic and post-meiotic cells than in either immature or mature testes (Fig. 1B, lanes 1–3).

Developmental Expression of Catalase (CAT) mRNA in the Mouse Testis

When CAT transcripts from mouse liver, brain, prepubertal testes, and sexually mature mouse testes were examined by northern blot hybridization, one CAT mRNA of approximately 2.4 kb was detected in both somatic and testicular cells (Fig. 2A, lanes 1–8). Higher levels of CAT mRNA were found in liver and brain than in testis (Fig. 2A, compare lanes 1 and 2 to lanes 3–8). As seen for GSHPx, additional slower migrating CAT transcripts were observed in the liver (Fig. 2A, lane 1). Lower levels of the major CAT transcript were observed in pachytene spermatocytes, round spermatids, and elongating spermatids (Fig. 2B, lanes 1–3) than in prepubertal or adult testes.

Developmental Expression of Manganese Superoxide Dismutase (SOD-2) mRNA in the Mouse Testis

The expression of SOD-2 mRNAs during male germ cell differentiation was examined by northern blot hybridiza-

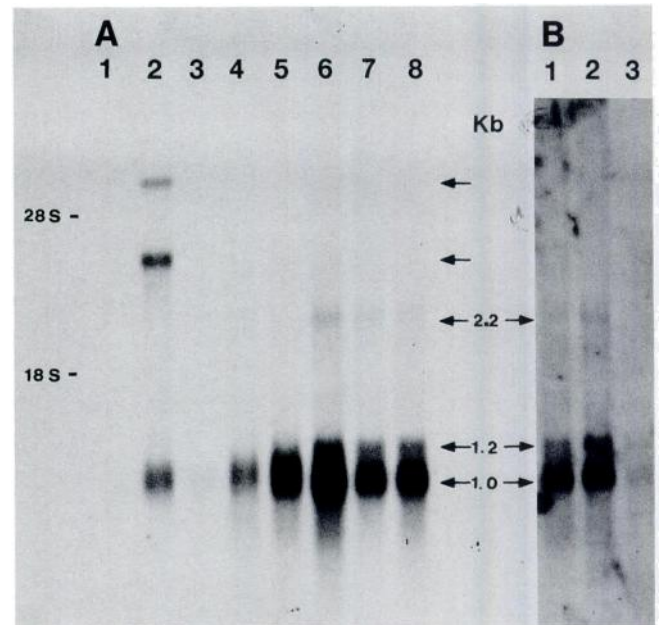


FIG. 3. Northern blot analysis of SOD-2 mRNAs in total RNA from mouse liver, brain, prepubertal testes, and adult testes. **(A)**, Aliquots of RNA (10 μ g) from adult mouse liver (lane 1), brain (lane 2), and testes from 6-day-old (lane 3), 12-day-old (lane 4), 17-day-old (lane 5), 22-day-old (lane 6), 25-day-old (lane 7), and adult (lane 8) mice were electrophoresed in 1% agarose gels and transferred to nylon membranes. The membranes were hybridized with a 32 P-labeled cDNA encoding human SOD-2. The arrows denote the positions of the multiple SOD-2 mRNAs. **(B)**, Aliquots of RNA (10 μ g) from pachytene spermatocytes (lane 1), round spermatids (lane 2), and elongating spermatids (lane 3) were analyzed as in A.

tions using a human SOD-2 cDNA probe that was 93% homologous to murine SOD-2 (Ho and Crapo, 1992). In contrast to the high levels of GSHPx and CAT mRNAs seen in the liver (Figs. 1A and 2A), SOD-2 mRNA expression in the liver was extremely low (Fig. 3A, lane 1). SOD-2 mRNA levels were barely detectable from testes of 6-day-old mice, at a developmental stage consisting of primarily somatic cells and mitotic germ cells (Fig. 3A, lane 3). An increasing amount of three SOD-2 transcripts with estimated sizes of 2.2, 1.2, and 1.0 kb was seen as the first wave of spermatogenesis proceeded (Fig. 3A, lanes 4–8). In testes of 12-day-old mice, a stage in which spermatogenesis has advanced to the start of meiosis, a SOD-2 mRNA of about 1.0 kb predominates (Fig. 3A, lane 4). Maximal expression of the three SOD-2 mRNAs was found in the testes of 22-day-old mice, at a developmental time when post-meiotic spermatids appear (Fig. 3A, lane 6). Lower levels of SOD-2 mRNAs are seen in RNA preparations from adult testis (Fig. 3A, lane 8). In the brain, in addition to the major 1.0-kb SOD-2 mRNA, two additional slower-migrating mRNA bands of about 3.0 and 4.5 kb were detected (Fig. 3A, lane 2).

When RNAs isolated from enriched populations of pachytene spermatocytes (meiotic cells), round sperma-

tids (early post-meiotic cells), and elongating spermatids (late post-meiotic cells) were analyzed by northern blotting (Fig. 3B), the highest levels of the three SOD-2 mRNAs of 2.2, 1.2, and 1.0 kb were detected in round spermatids (Fig. 3B, lanes 2). In agreement with the high levels of SOD-2 detected in testes from 22-day-old mice and a decrease in SOD-2 mRNA levels in adult testes (Fig. 3A, lanes 6 and 8), a marked decrease in the amount of SOD-2 mRNAs was observed in the elongating spermatids, the late-stage post-meiotic germ cells (Fig. 3B, lane 3). The reduced level of SOD-2 mRNAs in the elongating spermatid cell fraction is not due to RNA loss, because rehybridization of the same blot with a cDNA probe encoding SOD-1 revealed high levels of SOD-1 mRNA in this fraction (data not shown).

Polysomal Distribution of GSHPx, CAT, and SOD-2 mRNAs

To determine the relative translational status of the GSHPx, CAT, and SOD-2 mRNAs in mouse testis, aliquots of RNA samples prepared from post-mitochondrial supernatants of mature testes were fractionated in a 10% to 40% linear sucrose gradient and analyzed by northern blot hybridizations (Fig. 4). Previous studies with protamine and transition protein mRNAs have demonstrated that non-polysomal mRNAs are found under these centrifugation conditions in fractions 1–3, whereas fractions 4–6 contain mostly polysomal mRNAs (Kleene et al, 1984). The majority of the GSHPx mRNAs (Fig. 4A, lanes 4–6) and CAT mRNAs (Fig. 4B, lanes 5 and 6) were polysomal, indicating that both GSHPx and CAT mRNAs were efficiently translated. In contrast, when the same RNA blot was hybridized with the SOD-2 cDNA probe, a differential distribution of the two predominant SOD-2 mRNAs was seen. The 1.0-kb transcript was distributed approximately equally between the non-polysomal and polysomal fractions (Fig. 4C), whereas the 1.2-kb SOD-2 mRNA was present mostly in the non-polysomal fractions (Fig. 4C, lanes 1 and 2).

Discussion

We have previously analyzed SOD-1 mRNA expression and its translational regulation during mouse spermatogenesis (Gu et al, 1995a) and identified a testis-specific SOD-1 transcript that is transcribed in round spermatids from an alternative promoter and translationally regulated during spermiogenesis. Here we examine the expression of three additional antioxidant enzymes, GSHPx, CAT, and SOD-2, during mouse spermatogenesis. GSHPx and CAT mRNAs are expressed at high levels in the mouse testis throughout testicular development. In the testis we detect one predominant transcript of GSHPx and one tran-

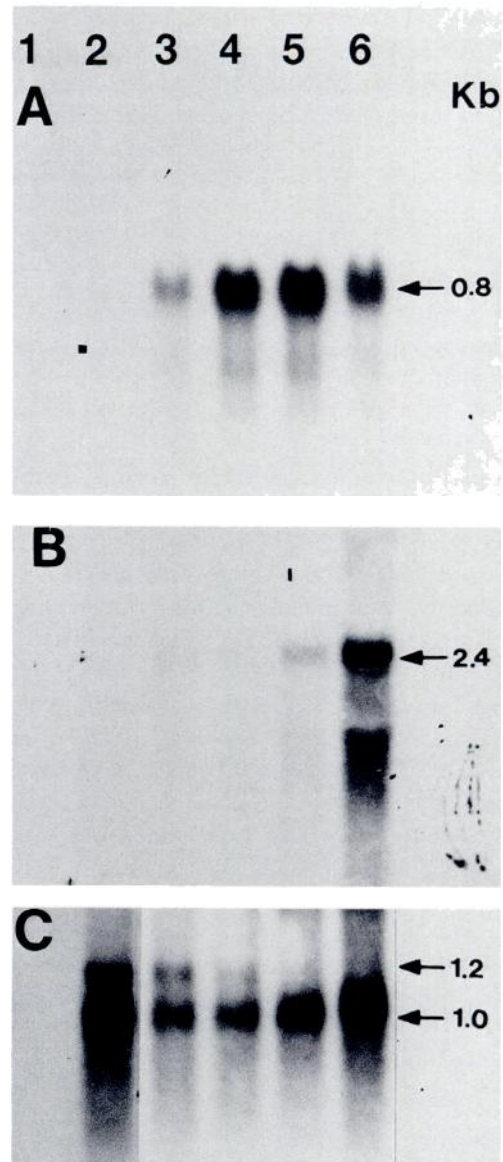


FIG. 4. Distribution of GSHPx, CAT, and SOD-2 mRNAs in a post-mitochondrial testicular extract fractionated by sucrose gradient centrifugation. Fraction 1 represents the top of the gradient. Equal volumes of RNA from each fraction were resolved in 1% agarose gels and blotted on nylon membranes. For each autoradiogram, the mRNA sizes are noted at the right. (A), Autoradiogram obtained after hybridization with the GSHPx cDNA. (B), Autoradiogram obtained after hybridization with the CAT cDNA probe. (C), After the CAT probe was removed by incubating the membrane in 50% formamide, 2x standard saline citrate, and 0.1% sodium dodecyl sulfate for 30 minutes at 70°C, the membrane was rehybridized with the SOD-2 cDNA probe.

script of CAT mRNA, whereas multiple transcripts of CAT and GSHPx mRNAs are observed in mouse liver (Fig. 1A, lane 1; Fig. 2A, lane 1). Meiotic and post-meiotic cells contain less GSHPx mRNA and CAT mRNA than prepuberal and adult mouse testes, suggesting that most of the testicular GSHPx and CAT mRNAs are present in the somatic cells of the testis. The high levels of

GSHPx or CAT transcripts in the immature and mature testes, their efficient translation (Fig. 4A and 4B), and the wide distribution of GSHPx and CAT enzymes in testicular cells (data not shown) suggest a biological importance of these enzymes in somatic and late stages of germ cells of the mammalian testis. Several reports have indicated that plasma membranes of testicular germ cells contain high levels of polyunsaturated fatty acids, which are extremely susceptible to free radical damage (Van Loon et al, 1991; Robinson et al, 1992; Griveau et al, 1995). The expression of GSHPx and CAT mRNAs we detected in the mammalian testis is in good agreement with the studies by Alvarez and Storey (1989) and Jeulin et al (1989), where they demonstrated that GSHPx and CAT proteins play a significant role in protecting spermatozoa from oxidative attack by removing hydrogen peroxide and lipid peroxide.

SOD-2, like SOD-1 (Jow et al, 1993; Gu et al, 1995a) is encoded by multiple transcripts. Although SOD-2 mRNA is encoded by a nuclear gene, the protein is primarily localized in mitochondria (Barra et al, 1984). Because the mitochondrial electron transport system produces endogenous O_2^- (Loschen et al, 1974), it has been suggested SOD-2 plays a crucial role in the protection of the organelle from oxidative damage (Slot et al, 1986). SOD-2 expression has been examined in many species. In human cell lines, two SOD-2 transcripts have been detected, a major mRNA of 1.3 kb and a minor mRNA of 4.2 kb (Ho and Crapo, 1992). Recent studies of SOD-2 mRNA expression in various murine tissues have revealed the presence of multiple SOD-2 transcripts with a predominant SOD-2 mRNA of about 1 kb (Jones et al, 1995). Mouse testis is one of the tissues where high levels of SOD-2 mRNA are expressed (Jones et al, 1995). Our data are in agreement with the report of Hurt et al (1992), where they have identified five different SOD-2 mRNAs in the rat. The quantity and ratio of the five transcripts vary among the tissues. The brain, kidney, and heart contain all five mRNAs, whereas liver, spleen, and testis contain primarily two of the SOD-2 mRNAs, a predominant one of 1.0 kb and a less abundant 1.3-kb transcript. We also detect five different SOD-2 transcripts in mouse tissues (Fig. 3A), three of which are expressed in mouse testis, a major 1.0-kb SOD-2 mRNA, a less abundant transcript of about 1.2 kb and a minor 2.2-kb transcript (Fig. 3A). The expression of SOD-2 mRNA is developmentally regulated with an undetectable level in the testes of 6-day-old mice and high levels in the testes of 22-day-old mice. Moreover, although significant levels of SOD-2 mRNAs are found in pachytene spermatocytes and round spermatids, elongating spermatids contain extremely low amounts of SOD-2 mRNAs (Fig. 3B, lane 3). We do not know whether the decreased level of SOD-2 mRNAs in

late spermatids is caused by changes in the stability of SOD-2 mRNA during spermiogenesis.

Mammalian cells contain two SOD enzymes, SOD-1 and SOD 2. Holland et al (1982) have reported that in rabbit spermatozoa, about 5% of the total SOD activity is contributed by SOD-2. In rat liver, about 7–10% of the total SOD activity has been estimated to be SOD-2 (Peters-Jori et al, 1975; Salin et al, 1978). Our previous studies of SOD-1 expression during spermatogenesis have identified three SOD-1 mRNAs of about 0.73, 0.80, and 0.93 kb (Gu et al, 1995a). The 0.93-kb mRNA is testis specific and is transcribed during mid-spermiogenesis and translated days later in elongating spermatids. Taken together, the near absence of the SOD-2 mRNAs in elongating spermatids and the subsequent translation of SOD-1 mRNA during late stages of spermatogenesis suggests that SOD-1 may functionally play a more important role than SOD-2 in the terminal stages of male germ cell differentiation.

The presence of transcripts on polysomes is generally indicative of mRNA being translated (Kleene, 1989). Although most of the GSHPx and CAT mRNAs are polysomal, the multiple SOD-2 mRNAs show different levels of translation efficiency. The 1.0-kb SOD-2 mRNA is equally distributed between the polysomal and non-polysomal fractions, whereas most of the 1.2-kb SOD-2 mRNA is non-polysomal, suggesting that their expression, like other germ cell-expressed genes such as the protamines and transition proteins, is translationally regulated (Kleene, 1989). Because multiple mammalian SOD-2 mRNAs have been reported to be generated by utilization of alternative polyadenylation sites (Ho and Crapo, 1992; Hurt et al, 1992; Jones et al, 1995), the extended 3' untranslated region (UTR) of the 1.2-kb SOD-2 mRNA may influence its low level of polysome formation. For SOD-1, an extended sequence in its 5' UTR regulates its translational efficiency (Gu et al, 1995a).

The regulation of superoxide dismutase in the testis is complex. Both SOD-1 and SOD-2 have multiple transcripts that show different levels of translational efficiency, i.e, a different distribution of translated (polysomal) and untranslated (non-polysomal) mRNAs. Our previous studies of the translational regulation of SOD-1 have demonstrated that the two shorter SOD-1 transcripts representing the somatic type SOD-1 mRNAs are efficiently translated, whereas most of the longest SOD-1 transcript, the 0.93-kb testis-specific mRNA, is in the non-polysomal fraction. Recently, we have identified a testicular protein that binds to the 5' UTR of the SOD-1 mRNA and represses its translation "in vitro" (Gu et al, 1995a; Gu and Hecht 1996). Of the mRNAs encoding the three antioxidant enzymes we examined here, the 1.2-kb SOD-2 mRNA and the 0.93-kb SOD-1 mRNA appear to be under

similar regulation. We know that overexpression of SOD-1 (Elroy-Stein et al, 1986; Avraham et al, 1988) or reduced SOD-1 activity levels is deleterious (Deng et al, 1993; Rosen et al, 1993). Preliminary studies indicate that rodent testicular cells contain a relatively constant level of both SOD-1 protein and SOD-1 enzymatic activity (Gu, unpublished data). Because SOD-1 and SOD-2 both catalyze the conversion of superoxide to hydrogen peroxide in cells, we propose that a similar translational control mechanism, perhaps also dependent upon specific RNA-protein interactions, may also be operating to prevent overproduction or underproduction of SOD-2 in the testis.

In conclusion, we find that GSHPx and CAT mRNAs are present throughout testis development and are efficiently translated, whereas the expression of SOD-2 mRNAs is both developmentally and translationally regulated during spermatogenesis. The differential expression of GSHPx, CAT, SOD-1, and SOD-2 mRNAs in the testis suggests that a regulated expression of antioxidant enzymes is necessary to optimally protect germ cells from oxidative stress.

References

- Aebi M. Catalase *in vitro*. *Methods Enzymol* 1984;105:121-126.
- Aitken RJ, Clarkson JS. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. *J Reprod Fertil* 1987;81:459-469.
- Aitken RJ, Clarkson JS, Fishel S. Generation of reactive oxygen species, lipid peroxidation and human sperm function. *Biol Reprod* 1989;40:183-197.
- Aitken RJ, Irvine DS, Wu FC. Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. *Am J Obstet Gynecol* 1991;164:542-551.
- Alcivar AA, Hake LE, Millette CM, Trasler JM, Hecht NB. Mitochondrial gene expression in male germ cells of the mouse. *Dev Biol* 1989;135:263-271.
- Alvarez JG, Storey BT. Role of glutathione peroxidase in protecting mammalian spermatozoa from loss of motility caused by spontaneous lipid peroxidation. *Gamete Res* 1989;23:77-90.
- Alvarez JG, Storey BT. Evidence for increased lipid peroxidative damage and loss of superoxide dismutase activity as a model of sublethal cryodamage to human sperm during cryopreservation. *J Androl* 1992;13:232-241.
- Alvarez JG, Touchstone JB, Blasco L, Storey BT. Spontaneous lipid peroxidation and production of hydrogen peroxide and superoxide in human spermatozoa: superoxide dismutase as major enzyme protectant against oxygen toxicity. *J Androl* 1987;8:338-348.
- Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative disease of aging. *Proc Natl Acad Sci USA* 1993;90:7915-7922.
- Avraham KB, Schickler M, Sapoznicov D, Yarom R, Groner Y. Down's syndrome: abnormal neuromuscular junction in tongue of transgenic mice with elevated level of human superoxide dismutase. *Cell* 1988;54:823-829.
- Barra D, Schinina ME, Simmaco M, Bannister JV, Bannister WH, Ratilio G, Bossa F. The primary structure of human liver manganese superoxide dismutase. *J Biol Chem* 1984;259:12595-12601.
- Bauche F, Fouchard M, Jégou B. Antioxidant system in rat testicular cells. *FEBS Lett* 1994;349:392-396.
- Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. *Physiol Rev* 1979;59:527-605.
- Crow JF. How much do you know about spontaneous human mutation rate? *Environ Mol Mutagen* 1993;21:122-129.
- Crapo JD, Oury T, Rabouille C, Slot JW, Chang L-Y. Copper, zinc superoxide dismutase is primarily a cytosolic protein in human cells. *Proc Natl Acad Sci USA* 1992;89:10404-10409.
- Davies KJA. Protein damage and degradation by oxygen radicals. *J Biol Chem* 1987;262:9895-9901.
- Deng H-X, Hentati A, Tainer JA, Iqbal Z, Cayabyab A, Hung WY, Getzoff ED, Hu P, Herzfeldt B, Roos RP, Warner C, Deng G, Soriano E, Smyth C, Parge HE, Roses AD, Hollewell RA, Pericak-Vouce MA, Siddique T. Amyotrophic lateral sclerosis and structural defects in Cu, Zn superoxide dismutase. *Science* 1993;261:1047.
- Dryja TP, Shizuo M, Petersen R, Rapaport JM, Walton D, Yandell DW. Parental origin of mutations of the retinoblastoma gene. *Nature* 1989;339:556-558.
- Elroy-Stein O, Bernstein Y, Groner Y. Overproduction of human superoxide dismutase in transfected cells: extenuation of paraquat-mediated cytotoxicity and enhancement of lipid peroxidation. *EMBO J* 1986;5:615-622.
- Fridovich I. The biology of oxygen radicals. *Science* 1978;201:875-880.
- Griveau JF, Dumont E, Renard P, Callegari JP, Lannou DL. Reactive oxygen species, lipid peroxidation and enzymatic defense systems in human spermatozoa. *J Reprod Fertil* 1995;103:17-26.
- Gu W, Hecht NB. Translation of a testis-specific Cu/Zn superoxide dismutase mRNA (SOD-1) is regulated by a 65 kDa which binds to its 5' untranslated region. *Mol Cell Biol* 1996; in press.
- Gu W, Hermo L, Hecht NB. In male mouse germ cells, copper-zinc superoxide dismutase utilizes alternative promoters that produce multiple transcripts with different translation potential. *J Biol Chem* 1995a;270:236-243.
- Gu W, Kwon YH, Oko R, Hermo L, Hecht NB. Poly(A) binding protein is bound to both stored and polysomal mRNAs in the mammalian testis. *Mol Reprod Dev* 1995b;40:273-285.
- Harman D. The aging process. *Proc Natl Acad Sci USA* 1981;78:7124-7128.
- Harman D. Free radical theory of aging. *Mutat Res* 1992;275:257-266.
- Ho Y-S, Crapo JD. Isolation and characterization of complementary DNA encoding human manganese-containing superoxide dismutase. *FEBS Lett* 1992;229:256-260.
- Holland MK, Alvarez JG, Storey BT. Production of superoxide and activity of superoxide dismutase in rabbit epididymal spermatozoa. *Biol Reprod* 1982;27:1109-1118.
- Hurt J, Hsu J-L, Dougall WC, Visner GA, Burr IM, Nick HS. Multiple mRNA species generated by alternative polyadenylation from the rat manganese superoxide dismutase gene. *Nucleic Acids Res* 1992;20:2985-2990.
- Jeulin C, Soufir JC, Weber P, Laval-Martin D, Calvayrac R. Catalase activity in human spermatozoa and seminal plasma. *Gamete Res* 1989;24:185-196.
- Jones PL, Kucera G, Gordon H, Boss JM. Cloning and characterization of the murine manganese superoxide dismutase-encoding gene. *Gene* 1995;153:155-161.
- Jow WW, Schlegel PN, Chicon Z, Philips D, Goldstein M, Bardin CW. Identification and localization of copper-zinc superoxide dismutase gene expression in rat testicular development. *J Androl* 1993;14:439-447.
- Kleene KC. Poly(A) shortening accompanies the activation of five mRNAs during spermatogenesis in the mouse. *Development* 1989;106:367-373.
- Kleene KC, Distel RJ, Hecht NB. Translational regulation and deadeny-

- lation of a protamine mRNA during spermatogenesis in the mouse. *Dev Biol* 1984;105:71-79.
- Loschen G, Azzi A, Richter C, Flohe L. Superoxide radicals as precursors of mitochondrial hydrogen peroxide. *FEBS Lett* 1974;42:68-72.
- Mastuda Y, Seki N, Utsugi-Takeuchi T, Tobar I. X-ray and mitomycin C (MMC)-induced chromosome aberrations in spermatogenic germ cells and the repair capacity of mouse eggs for X-ray and MMC damage. *Mutat Res* 1989;211:67-75.
- Menella MR, Jones R. Properties of spermatozoal superoxide dismutase and lack of involvement of superoxides in metal ion catalyzed lipid-peroxidation reactions in semen. *Biochem J* 1980;191:289-297.
- Nanogaki T, Noda Y, Narimoto K, Shiotani M, Mori T, Matsuda T, Yoshida O. Localization of Cu/Zn-superoxide dismutase in the human male genital organs. *Hum Reprod* 1992;7:81-85.
- National Research Council. *Health Effects of Exposure to Low Levels of Ionizing Radiation*. BEIV V. Washington, DC: National Academy Press; 1990.
- Peskin AV, Shlyahova L. Cell nuclei generate DNA-nicking superoxide radicals. *FEBS Lett* 1986;194:317-321.
- Petters-Jori C, Vandervoorde A, Baudhuin P. Subcellular localization of superoxide dismutase in rat liver. *Biochem J* 1975;150:131-139.
- Robinson BS, Johnson DW, Poulos A. Novel molecular species of sphingomyelin containing 2-hydroxylated polyenic very long chain fatty acids in mammalian testes and spermatozoa. *J Biol Chem* 1992;267:1746-1751.
- Rosen DR, Siddique T, David P, Figlewicz DA, Sapp P, Donaldson D, Goto JP, Deng H-X, Rahmani Z, Krizus A, McKenna-Yasek D, Cayabyab A, Gaston SM, Berger R, Tanzi E, Halperin JJ, Herzfeldt B, Van der Bergh R, Hong WY, Bird T, Deng G, Mulder DW, Smyth C, Liang NG, Gusella JS, Horvitz HR, Brown RH Jr. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993;362:59-62.
- Salin ML, Day ED, Crapo JD. Isolation and characterization of a manganese containing superoxide dismutase for rat liver. *Arch Biochem Biophys* 1978;187:223-228.
- Slot J, Geuze H, Freeman B, Crapo JD. Intracellular localization of the copper-zinc and manganese superoxide dismutase in rat liver parenchymal cells. *J Lab Invest* 1986;55:363-371.
- Stadtman ER. Protein oxidation and aging. *Science* 1992;257:1220-1224.
- Van Loon AA, Boer PJ, Van der Schans GP, Mackenbach P, Grootegoed JA, Baan RA, Lohman PHM. Immunochemical detection of DNA damage induction and repair at different cellular stages of spermatogenesis of the hamster after *in vitro* or *in vivo* exposure to ionizing radiation. *Exp Cell Res* 1991;193:303-309.
- Vogel F, Rathenberg R. Review: spontaneous mutation in man. *Adv Hum Genet* 1975;5:223-318.
- Wagner JR, Hu CC, Ames BN. Endogenous oxidative damage of deoxycytidine in DNA. *Proc Natl Acad Sci USA* 1992;89:3380-3384.