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Unveiling the Molecular Mechanisms Behind Selenium-Related Diseases Through Knockout Mouse Studies

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Abstract

Selenium (Se), in the form of the 21st amino acid selenocysteine, is an integral part of selenoproteins and essential for mammals. While a large number of health claims for Se has been proposed in a diverse set of diseases, little is known about the precise molecular mechanisms and the physiological roles of selenoproteins. With the recent and rigorous application of reverse genetics in the mouse, great strides have been made to address this on a more molecular level. In this review, we focus on results obtained from the application of mouse molecular genetics in mouse physiology and discuss these insights into the physiological actions of selenoproteins in light of evidence from human genetics. *Antioxid. Redox Signal.* 12, 851–865.

Introduction

The essential trace element selenium (Se), in the form of the 21st amino acid selenocysteine (Sec), is an integral part of about two dozen selenoproteins in mammals. Nowadays, it is well established that Se exerts its main biological functions through selenoproteins, which are characterized by carrying one or several Sec residue(s). It is assumed that Sec essentially contributes to enzyme catalysis, but this has been demonstrated only for some selenoproteins. Owing to structural and catalytic homologies, some of the selenoproteins can be classified into the glutathione peroxidase (GPx), deiodinase (Dio), and thioredoxin reductase (Txnrd) families of proteins, while others, including SePP, appear to be unique with regard to structure and function.

Mammals express eight glutathione peroxidases, however, only cytosolic GPx (GPx1), gastrointestinal GPx (GPx2), plasma GPx (GPx3), GPx4 (also referred to as phospholipid hydroperoxide glutathione peroxidase, PHGPx), and olfactory epithelium GPx6 are selenoproteins in humans, while GPx6 Sec is replaced by cysteine in rodents (57). Like GPx5, which is predominantly expressed in epididymis, GPx7 and GPx8 are cysteine-containing enzymes. Deiodinases (Dio) are enzymes capable of removing iodine atoms from iodothyronines. Depending on the Dio isoenzyme, the thyroid al prohormone thyroxine (T4) is deiodinized to yield nuclear receptor-binding 3,3′,5-triiodothyronine (T3) or inactive iodothyronines. Dio enzymes are therefore integral parts of the thyroid hormone axis and modulate thyroid hormone action by local activation and inactivation (93). All three mammalian thioredoxin reductases are selenoproteins, where the Sec moiety sits as the penultimate amino acid on the highly flexible C-terminal tail (42). This structural feature is believed to account for the high versatility of thioredoxin reductase catalysis (43). Selenoprotein P (SePP) is unusual as it contains 10–17 Sec residues, depending on the vertebrate species. The high Sec content is likely related to the Se-transport role of SePP in plasma. Since most plasma Se is contained in SePP, SePP serves as a biomarker for Se status of the body (9). Beside these, SeP15 and the closely related SePM have been implicated in protein folding in the endoplasmic reticulum (32). Similarly, SelS is a component of the ER-associated degradation machinery and is associated with circulating inflammatory cytokine levels (116). The rather small selenoprotein SelW, which is preferentially expressed in heart (except rodents), muscle, spleen, and brain, binds glutathione (GSH) and is assumed to confer redox functions, although the physiological function of SelW remains largely obscure (112).

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SelN is an ER-resident selenoprotein that associates with the muscular ryanodine receptor and is essential for correct muscle function. Its mutation in human patients is underlying a family of congenital muscular diseases (55, 63).

Limited availability of nutritional Se or severe Se depletion, either alone or in combination with other trace elements and/or other environmental factors, have been considered the cause of, or modulator, for a plethora of diseases in humans, including cardiovascular disease, cancer, immune system dysfunction, neurodegeneration, and male infertility (75). Thus, it was somewhat surprising when only 25 genes encoding selenoproteins were described in humans. This would suggest that the impaired expression of some of these selenoproteins in different tissues, due to perturbed Se uptake, or genetic polymorphisms, could be an important factor for all these diseases. On the other hand, it has been known for a long time that plasma Se levels affect the expression of individual selenoproteins distinctively, and that different tissues exhibit specific demands for Se—indeed, this feature has been frequently referred to as 'the hierarchy of selenoproteins'. In other words, expression of some selenoproteins, including GPx2, GPx4, and Txnrd, is maintained even under prolonged selenium deprivation, while the expression of other selenoenzymes, including GPx1, GPx3, and SePP, swiftly responds to varying plasma Se concentrations (4, 44). The same holds true for the retention of Se in different organs after experimental or nutritional Se depletion (3). For instance, in brain and testis Se levels are strongly maintained, followed by kidney and cardiac muscle, while liver appears to be highly dependent on dietary Se supply. These findings have been reviewed recently in more detail (85).

With regard to this remarkably high complexity, it has remained quite difficult to assign Se-related human diseases to individual selenoproteins. This is even further complicated by the fact that some of the selenoproteins confer equal or at least similar functions, and that they are expressed in a tissue- and cell-type-specific manner. Also, the intricate and intertwined functions of selenoproteins and nonselenoproteins, for instance to maintain cellular redox balance or to control hormone metabolism, adds another layer of complexity towards a better understanding of selenoprotein function in physiology and disease development. And as selenoproteins are usually expressed in low levels due to the very inefficient decoding of the UGA codon as Sec, which requires the concerted action of cis- and trans-acting factors (103), the molecular investigation of these proteins in cells and tissues represents another major drawback at least for practical reasons.

With the help of mouse genetics, however, a great leap has been made within the last couple of years towards a clearer picture concerning the role of individual selenoproteins in embryo and tissue development and even in cell signaling pathways. However, as only approximately a dozen of the selenoprotein genes have been genetically targeted thus far (Table 1), future studies using transgenic mice will yield novel insights into selenoprotein functions. In parallel to this, the recent boost in genome-wide association studies in humans will certainly unveil novel mutations in specific selenoproteins, which either predispose or protect from certain diseases. The first mutations in a specific selenoprotein linked to a genetic disorder were shown for SelN, which is associated with rigid spine muscular dystrophy (63).

Here, we summarize the major findings, obtained mainly by transgenic studies in mice, which have been instrumental in uncovering the role of Se in tissue development and function, and particularly in light of the contribution of Se shortage to tissue malfunction and disease development (Fig. 1). This review is structured as such that tissues with seemingly less obligation for Se will be discussed initially, whereas tissues which are ranked high in the hierarchy of Se requirement will be discussed thereafter.

Hepatic System

The liver is central for Se metabolism of the body. Dietary Se taken up in the intestine is rapidly extracted by the liver. There it is used for selenoprotein biosynthesis, including SePP, which represents the main plasma Se transport protein. SePP has been genetically inactivated in the mouse by two groups with identical findings (48, 86). While hepatic Se levels remained normal or increased slightly upon *Sepp*-inactivation, tissue Se content in kidney, muscle, testis, brain, and plasma Se decreased sharply. At the time, the most striking effect was the significant decrease in brain Se, a finding never achieved by dietary Se deficiency alone. Moreover, tissue Se deficiency revealed those organs which rely on Se delivery by SePP. Since selenoenzymes require Se for their biosynthesis, activities of GPx and Txnrd were reduced in *Sepp*-deficient mice. Similarly, genetic deletion of only the Se-rich C-terminus of *Sepp* supported the Se transport role of SePP *in vivo* (47).

Hepatic Se may also enter the methionine pool as selenomethionine and thus may be incorporated into albumin. This Se may raise plasma Se levels, but does not represent an immediately bioavailable source of Se. Dietary selenomethionine is expected to enter the hepatic trans-sulfuration pathway leading to the formation of Sec. Of note, Sec is not directly incorporated into proteins. Rather, selenocysteine lyase liberates a Se atom from Sec and provides it to selenophosphate synthetase (SPS2) for synthesizing selenophosphate. Selenophosphate is then used by selenocysteine synthase (SecS) to convert Ser-tRNA$^{[Ser]Sec}$ into Sec-tRNA$^{[Ser]Sec}$ (114).

A mouse model in which hepatic selenoprotein biosynthesis is abrogated by genetic inactivation of tRNA$^{[Ser]Sec}$ (gene symbol *Trsp*) demonstrated that hepatic SePP biosynthesis is important for whole body Se metabolism in mice (92). Interestingly, these mice were a biochemical phenocopy of *Sepp*-deficient mice with regard to plasma Se, kidney Se content, and selenoprotein expression. However, brain Se content was preserved in these mice, pointing to certain autonomy of the brain with respect to Se metabolism.

Hepatocytes can tolerate the complete loss of selenoprotein biosynthesis (92, 101), although under different dietary conditions they suffered hepatic degeneration (14). These animals also showed changes in hepatic expression of lipoproteins and cholesterol biosynthetic enzymes (95). Liver is also the expected site of formation of selenosugars which are thought to represent a physiological route of Se excretion via the kidney. Accordingly, *Sepp*-deficient mice displayed increased selenosugar excretion, possibly a sign of hepatic Se overflow (11).

Kidney

Relatively little is known concerning the specific role of an individual selenoprotein in kidney function, although numerous previous studies revealed the expression of various

Table 1. Summary of Specific Selenoprotein Knockout Mice

Gene	*Authors*	*Approach*	*Major physiologic phenotype*	*Ref.*
GPx1	Ho *et al.*, 1997	Complete	No spontaneous phenotype, but susceptible to ischemic and toxic insults (reviewed in (90))	(50)
GPx2	Esworthy *et al.*, 2000	Complete	No spontaneous phenotype	(29, 30)
GPx1/GPx2	Esworthy *et al.*, 2001	Complete	Double knockout mice develop colitis (dependent on gut flora)	(15, 29)
GPx4	Yant *et al.*, 2003; Imai *et al.*, 2003; Garry *et al.*, 2008; Seiler *et al.*, 2008	Complete	Embryonic lethality at E7.5	(115) (52) (39) (94)
GPx4	Seiler *et al.*, 2008	Conditional	Neurodegeneration in hippocampus and cortex	(94)
nGPx4	Conrad *et al.*, 2005	Complete	Fully viable; delayed sperm chromatin condensation	(19)
mGPx4	Schneider *et al.*, 2009	Complete	Fully viable; male infertility	(80)
Txnrd1	Jakupoglu *et al.*, 2005; Bondareva *et al.*, 2007	Complete	Embryonic lethality between E8.5 and E10.5	(7, 54)
Txnrd1	Jakupoglu *et al.*, 2005; Soerensen *et al.*, 2008	Conditional	No phenotype in heart; cerebellar hypoplasia	(54, 99)
Txnrd2	Conrad *et al.*, 2004	Complete	Embryonic lethality between E13.5 and E15.5	(18)
Txnrd2	Conrad *et al.*, 2004; Geisberger *et al.*, 2007; Soerensen *et al.*, 2008	Conditional	Congestive heart failure and postnatal death; no defect in B-cell, T-cell or brain development	(18, 41, 99)
Txnrd3	Not reported			
Dio1	Schneider *et al.*, 2006	Complete	Increased fecal iodine excretion, minor changes among circulating thyroid hormones (T4, rT3)	(83)
Dio2	Schneider *et al.*, 2001 Ng *et al.*, 2004	Complete	Pituitary resistance to T4, impaired cochlear development, deafness	(66, 82)
Dio3	Hernandez *et al.*, 2006 Ng *et al.*, 2009	Complete	Central dysregulation of thyroid hormone axis; Premature cochlear development	(46, 67)
Dio1/Dio2	Galton *et al.*, 2009	Complete	Surprisingly little phenotype, slight behavioural changes, not similar to hypothyroid animals	(37)
SePP	Hill *et al.*, 2003; Schomburg *et al.*, 2003	Complete	Disturbed Se trafficking in the body, male infertility, low brain Se, neurological deficits, degeneration	(48) (86)
	Hill *et al.*, 2007	C-terminus	Reduced Se transport to testis and brain, but less severe than complete Sepp-deletion	(47)
SelR	Fomenko *et al.*, 2009	Complete	No spontaneous phenotype	(35)
Sep15 SelH SelI SelK SelM SelN SelO SelS SelT SelV SelW SPS2			Some of the corresponding genes have been inactivated in different laboratories and the phenotypes are currently under study.	

selenoproteins, such Dio, Txnrd, and GPx in kidney. Moreover, studies by Burk's laboratory pointed to an important role for Se in this tissue, as Se deficiency and concomitant GSH deprivation, induced by the GSH depletor phorone, caused severe liver and kidney injury (10). Surprisingly, podocyte-specific ablation of *Trsp* did not cause any histopathological phenotype in kidney (6). Even the treatment of these mice with streptozotocin as a diabetic model did not impact on body weight, blood glucose levels, and urinary albumin/creatinine ratios. Also, no changes in oxidative stress markers were detectable in the glomeruli of these mice, which is in fact unexpected as oxidative stress has been linked to the

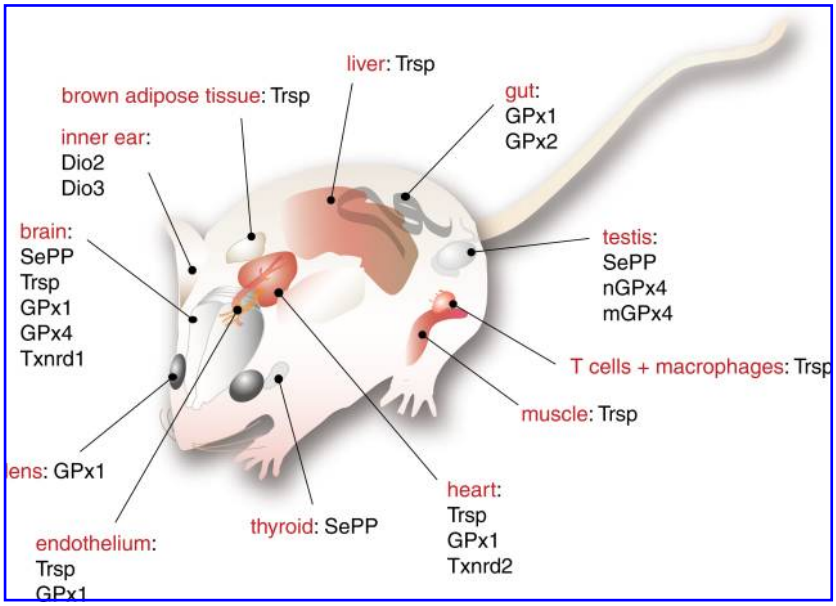

FIG. 1. Overview of adult organs and tissues affected by loss of distinct selenoproteins. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article at www.liebertonline.com/ars).

pathogenesis of diabetic nephropathy. Along the same line, de Haan *et al.* reported that *Gpx1* knockout mice are not more susceptible to the development of renal oxidative stress and nephropathy in a model of streptozotocin-induced diabetic nephropathy than wild-type mice (23), which was surprising as GPx1 is highly expressed in kidney and accounts for up to 96% kidney GPx activity.

The kidney also contributes to whole-body Se metabolism not only as the site of selenosugar excretion, but also as the source of circulating plasma glutathione peroxidase (GPx3). Although the generation of *Gpx3*-deficient mice has been presented orally by Dr. Raymond Burk, there is no publication of possible effects on the animals' health. Recently, it was demonstrated that SePP binds to apical renal tubular membrane in a megalin-dependent fashion (69). Megalin is a major endocytic receptor highly expressed in the kidney and involved in the re-uptake of filtrated plasma proteins, including Vitamin D-binding globulin and others. We have recently gathered evidence that body Se status is significantly decreased in *megalin*-deficient mice, which lose significant amounts of SePP through the urine (J Chiu and US, unpublished). Thus, a significant proportion of plasma SePP passes through the primary urine, is taken up in the proximal tubule, and contributes to circulating Se.

Bone, Cartilage, and Kashin–Beck Disease

Kashin–Beck disease is a degenerative osteoarticular disorder endemic to certain areas of Tibet, other parts of China, North Korea, and Siberia, where selenium levels in soil are very poor. Osteoathropathy usually becomes evident between 5 and 15 years of age. Subjects suffering from Kashin–Beck disease display different pathologies, which in the mild case range from joint deformation and impaired joint mobility and in the more severe cases to necrosis of growth plate, leading to decreased limb length and short stature. Investigations in rural Tibetan regions eventually revealed that combined Se- and iodine-deficiency are main risk factors for Kashin–Beck disease (64). More recently, other culprits have been implied in this disease, including fungal mycotoxins, which may either inhibit proteoglycan synthesis of chondrocytes or even trigger apoptosis of chondrocytes, possibly by a mechanisms involving the generation of free radicals, contaminated drinking water, and low amounts of vitamins A, D, and E (100). Although Se deficiency is certainly a risk determinant in this disease, looking at the current literature concerning knockout studies of individual selenoproteins in the context of bone/cartilage physiology is daunting.

Just recently, Downey and colleagues showed that osteochondroprogenitor-specific disruption of selenoprotein expression by using the $Trsp^{fl/fl}$ and *Col2a1-Cre* mice causes symptoms reminiscent of Kashin–Beck disease (27). Already one week after birth, the mutant mice displayed dwarfism, auricular hypoplasia, shortened snouts, reduced head size, and smaller limbs and tails. Moribund animals showed inspiratory respiratory distress, and the knockout mice barely survived longer than 4 to 5 weeks. Moreover, the size of the knees was reduced and the bones were shown to be unevenly ossified. Chondronecrosis, another hallmark of Kashin–Beck disease, was detectable in ears, trachea, and the articular cartilage. Hence, this model nicely demonstrates that murine skeletogenesis and maintenance of cartilage tissues requires proper selenoprotein expression (27), strongly supporting the idea that selenium deficiency is a major contributory factor on Keshan–Beck disease. On the other hand, more tissue-specific knockout studies, including GPx4, Txnrd1, and Txnrd2, are needed to ultimately decipher the role of distinctive selenoproteins in the etiology of Kashin–Beck disease.

Cardiovascular System

Probably the most known human disease associated with Se-deficiency is Keshan disease, a cardiomyopathy endemic

in certain rural areas of China where the selenium content was found to be low in foods (40). Keshan disease is characterized by multifocal necrosis, fibrous replacement, and calcification of the myocardium, myocytolysis, and an enlarged myocardium. The major changes are mainly found in mitochondria and appear to be most important in the development of myocardial lesions. Cardiomyocytes reveal swollen mitochondria with stretched cristae and a severely compromised overall activity of oxidative phosphorylation. Keshan disease is not only caused by Se deficiency. The etiology of this disease also appears to be associated with a viral infection; in mice it was shown that Se deficiency promotes the conversion of the virus from a nonpathogenic variant into a pathogenic form (2). This was nicely elucidated in Se-deficient, vitamin E-deficient or *Gpx1* knockout mice. In the coxsackievirus-induced myocarditis model, a benign strain of coxsackievirus B3 virus was shown to become virulent, and this change in the viral phenotype was linked to point mutations in the viral genome most likely due to increased oxidative stress in the different mice. Subsequently, it was shown that *Gpx1*-deficient mice showed a strong impairment in cardiac function in response to doxorubicin treatment (38). This was evidenced by reduced contractility and diastolic values, decreased heart rates, and lowered coronary flow rate, indicating that GPx1 protects cardiac tissue from oxidative stress.

Another selenoprotein, highly expressed in cardiac tissue, is mitochondrial thioredoxin reductase (Txnrd2). Targeted disruption of *Txnrd2* in mice causes embryonic lethality at the fetal stage (18). Knockout embryos were slightly reduced in overall size, but they were highly anemic, which indicated perturbed fetal hematopoiesis and/or defective heart development. In fact, there was a strong increase in the number of hematopoietic cells in liver and a reduced proliferation of cardiac cells. Cardiac tissue-restricted inactivation of *Txnrd2* caused congestive heart failure and postnatal death, further highlighting the importance of Txnrd2 in heart function. Ultrastructural analysis revealed massive swelling of cardiomyocytic mitochondria and loss of mitochondrial structures, including extended cristae. As previously mentioned, these histopathological changes are also manifested in Keshan disease patients. *Ex vivo* studies with $Txnrd2^{-/-}$ fibroblasts showed that proliferation was compromised and that knockout cells were highly sensitive to GSH depletion, which indicated that the two major antioxidant systems work cooperatively to control intracellular levels of oxygen radicals. On the other hand, cytosolic thioredoxin reductase (Txnrd1) was shown to play a negligible role both in embryonic and adult heart physiology (54). Hence, it must be concluded that Txnrd2 is of paramount importance for cardiac development and function and that its impaired expression in Se shortage may be linked to the cardiomyopathy in Keshan disease.

With regard to cardiac tissue, comparably little is known regarding individual selenoproteins in the vascular system, except for GPx1. Endothelium-specific deletion of *Trsp* using the *Tie2-Cre* mouse line caused an overall poorly developed vascular system and major developmental abnormalities in limbs, tail, and head from E12.5 onwards—eventually $Trsp^{-/-}$ mice died before birth (98). More specifically, brain and spinal cord of the knockout embryos appeared to be largely necrotic, and there was a decreased number of mature erythrocytes and extensive multifocal hemorrhage. Interestingly, endothelial cells in the aorta showed hypertrophy.

To date, the only selenoenzyme analyzed in endothelium in more detail is GPx1. Nitrosative and oxidative stress caused vascular dysfunction in homozygous *Gpx1* null mice, as shown by studying the vasodilating effects (*e.g.*, in response to bradykinin) (36). Conversely, *Gpx1* transgenic mice on a heterozygous cystathione ß-synthase null background were protected from hyperhomocyst(e)inemia, a risk factor of atherosclerosis, presumably by increasing bioavailable nitric oxide (NO) (111). When crossed to the *apolipoprotein E (apoE)*-deficient mice and fed for 24 weeks a Western-type diet, *Gpx1* knockout mice developed more atherosclerotic lesions compared to control mice (104) and to mice deficient only in *Gpx1* but wild-type for *apoE* (24). Although ROS levels were increased in these mice, no differences in lipoprotein oxidation could be detected. Yet GPx1 confers antiatherogenic functions, and a reduced "red-cell" GPx1 activity in humans has been associated with an augmented hazard ratio in cardiovascular events (5). Despite these studies, it remains to be elucidated whether other selenoproteins are essential for the proper development and function of the vascular system.

Hematopoietic System and Immune Function

Beneficial effects of dietary Se on the immune function have been reported to some extent, although molecular insights regarding the role of individual selenoproteins in the immune system are still scarce (reviewed in (75)). For instance, Se deficiency has been linked with impaired immunocompetence in humans, whereas supplementation was immunostimulant as measured by the proliferation rate of activated T cells and natural killer cell activity. By contrast, Se shortage was associated with the occurrence, virulence, and disease progression of some viruses, as described above.

Surprisingly little is known, however, about the role of selenium or selenoproteins at molecular level in immune function. Mice specifically lacking *Txnrd2* were shown to suffer from severe anaemia and embryonic death at the fetal stage (18). One major contributory factor for the anemic phenotype was massively augmented apoptosis of hematopoietic cells and most likely impeded growth of hematopoietic stem cells as demonstrated by colony-forming-unit assays *ex vivo*. However, B- and T-cell-specific disruption of *Txnrd2* did not impact on the development of lymphoid cells (41).

Abrogation of selenoprotein expression in T cells using the *Lck-Cre* mouse and the $Trsp^{fl/fl}$ mice caused partial atrophy of spleen, thymus, and lymph nodes, accompanied with an overall reduction of splenic $CD3^+$ and $CD4^+$ T cells (97). In particular, $CD8^+$ cells were more affected than $CD4^+$ cells. Selenoprotein-less T cells also displayed strongly reduced responsiveness to T cell receptor stimulation due to impaired IL-2 receptor induction and the lack of ERK signaling. Moreover, it was also shown that knockout of all selenoproteins in T cells had higher basal levels of endogenous ROS and that the defective proliferation of the knockout T-cells in response to CD3 and CD28 could be restored to wild-type levels in the presence of the thiol-containing antioxidant N-acetylcysteine (97). This strongly suggests that selenoproteins are indeed important for proper T cell function. On the other hand, Se deficiency rendered BALB/c mice entirely refractory to pristane induction of peritoneal plasmacytomas, the prime experimental model of inflammation-dependent plasma cell transformation (31). The cellular mechanism was

postulated to be compromised responsiveness of Se-deprived monocytes and neutrophils to chemoattractants, including thioredoxin and chemokines. Needless to say, many questions regarding the precise role of Se in immune function still remain elusive, and the role of Se in the immune system might be more complex than previously assumed.

Gastrointestinal Tract and Prostate Cancer

Major interest in the possible role of Se in gastrointestinal and prostatic cancer was sparked by the Nutritional Prevention of Cancer (NPC) Trial (16). In this study, Se supplementation (200 μg Se daily) over 5 years was able to significantly decrease the risk ratio for developing prostatic cancer, colon cancer, and total cancer mortality. This and other data, including clinical and animal models of intestinal cancer, were the basis for a large scale intervention trial testing the possible benefit regarding cancer incidence of Se and vitamin E supplementation in 32,000 healthy men over a 12-year period (SELECT: SELenium and vitamin E in Cancer prevention Trial (56)). While the NPC trial had demonstrated a benefit only for those subjects that entered the study with lower than 105 μg Se/L plasma, this variable was not used to stratify the subjects in the SELECT trial, with the effect that only few fell in the low-Se group. Accordingly, the SELECT trial was terminated in 2009 because of lack of efficacy after a median period of only 5.45 years (59). Thus, it remains unclear whether the failure of Se to lower cancer risk in the SELECT trial is a result of weakness in trial design or has unveiled the weaknesses of prior studies. While animal experiments have already supported the cancer-preventive action of Se supplements in several models, there is no consensus regarding the mode of Se action. While some researchers assume specific chemopreventive activities of certain natural or synthetic selenocompounds, others favor the hypothesis that Se acts through selenoproteins. Therefore, several studies have been initiated to analyze the possible role of specific selenoproteins in gastrointestinal cancer models utilizing selenoprotein-deficient transgenic mouse models.

Gastrointestinal (GI-)Gpx (GPx2) is mainly expressed in the intestinal tract. Targeted disruption of its gene, however, was not reported to lead to any obvious phenotype. When mice deficient in both *Gpx1* and *Gpx2* were generated, they spontaneously developed colitis (29), which was caused by intestinal helicobacter, an established risk factor for intestinal neoplasia in man. The same authors also showed that $Gpx1^{+/-}/Gpx2^{-/-}$ mice develop ileocolitis when raised on a Se-deficient diet, while a single functional allele of *Gpx2* completely prevented this inflammatory disorder even in $Gpx1^{-/-}$ mice (30). Thus, GPx2 may play a pivotal role in protection of the intestine and contribute decisively to the chemopreventive potential of Se against colon cancer.

The *Apc (Min)* mouse model develops spontaneous adenomas in the small intestine. These mice are a model for familial adenomatous polyposis coli (FAP), a congenital disease in humans who are haplo-insufficient for the *APC* gene. Se supplementation significantly reduced the number of tumors and the tumor burden per animal (22). Since this model does not rely on the somewhat artificial induction of tumors by chemical carcinogens, this study implies that irrespective of the reason for tumor initiation, Se supplementation may be beneficial for protection from intestinal cancer.

In an attempt to test whether selenoproteins are involved in protection from intestinal tumorigenesis, mice with a mutated (partially dominant negative) $tRNA^{[Ser]Sec}$ transgene were subjected to treatment with the chemical carcinogen azoxymethane (53). Genetic interference with and reduction of selenoprotein expression in the intestine increased the number of aberrant crypt foci, preneoplastic lesions in the mucosa. These results are in line with a genetic model of prostate carcinogenesis, in which these tRNA mutant mice also suffered from accelerated tumor progression (26). Thus, transgenic mouse models strongly support a role of selenoproteins in preventing cancer development or slowing its progression. Clearly, more selenoproteins need to be studied in such models, and many transgenic and loss-of-function models are now available (Table 1).

Thyroid and Thyroid Hormone Axis

There have been claims in the clinical literature that thyroid pathology may be exacerbated by Se deficiency, but we did not find signs of pathology or functional impairment in thyroids from *Sepp*-deficient mice (84). The thyroid gland expresses quite a number of selenoproteins and the persistent production of hydrogen peroxide for thyroid hormone biosynthesis suggests that appropriate protective enzyme systems should be in place (88). We have therefore analyzed conditional thyroid-specific selenoprotein (*Trsp*)-deficient animals (J. Chiu and U. Schweizer, *unpublished*). The thyroids of these mice do not degenerate, as might have been expected, and there are no signs of functional impairments regarding thyroid hormone or thyreotropin-stimulating-hormone (TSH) levels, although thyroidal selenoenzyme levels are greatly decreased.

There is more evidence supporting the role of Se for thyroid hormone metabolism. The finding that thyroid hormone levels are changed in Se-deficient rats and hepatic deiodinase activity depends on dietary Se has led the path to the identification of type I deiodinase (Dio1) as a selenoprotein. By homology, type II (Dio2) and type III (Dio3) deiodinases were cloned. Dio2 is a 5′-deiodinase and thus can activate T4 to produce T3. Accordingly, the brain increases Dio2 expression during hypothyroidism in order to maintain sufficient brain T3 levels. Dio3 is a 5′-deiodinase, which inactivates both T4 and T3. Thus, tissues can protect themselves from T3 in order to prevent the pro-differentiation signal of T3. Dio1 is capable of both 5′- and 5-deiodination and thus was expected to contribute to both activation and inactivation of thyroid hormones. The manner in which the enzyme activates or inactivates the hormone remained elusive. Targeted inactivation of *Dio1* in the mouse did not alter significantly circulating thyroid hormone levels, irrespective of general (83) or liver-specific deletion (101). The short answer for our hypothesis here is that hepatic Dio1 may be mainly involved in degradation of thyroid hormones and liberation of iodine, before the thyronine backbone is excreted. This function is then compatible with the dual activity of the enzyme.

Dio2 is expressed in the pituitary and is part of the hypophyseal T4 sensor. Consequently, targeted inactivation of Dio2 resulted in de-repression of pituitary TSH by T4 and pituitary hyperthyroidism (82). In addition, several other developmental phenotypes have been observed, such as inner ear defects (66).

Targeted inactivation of *Dio3* resulted in several phenotypes wherein the most pronounced was central hypothyroidism (46). During the time when the hypothalamic setpoint for thyrotropin-releasing-hormone (TRH) (as regulator of TSH and ultimately T3 and T4) is fixed, the hypothalamus normally expresses significant Dio3 activity resulting in a protection from excess T3 and T4. In the absence of Dio3, the hypothalamus becomes hyperthyroid with the result of repression of TRH and establishment of a low TRH setpoint. Ultimately, the TRH response of the hypothalamus remains insufficient and central hypothyroidism is the paradoxical result.

Surprisingly, mice lacking both Dio1 and Dio2 still show little phenotype (37), indicating that the thyroid hormone axis is apparently quite stable in response to the deletion of *Dio* genes. It was therefore unexpected that patients carrying hypomorphic alleles of selenocysteine insertion sequence-binding protein 2 (*SECISBP2)* presented in the clinics because of aberrations of their thyroid hormone axes (28). SECISBP2 recognizes the selenocysteine insertion sequence (SECIS) element located in the 3′-untranslated region (3′-UTR) of selenoprotein mRNA and mediates the co-translational incorporation of Sec. While several accessible markers for selenoprotein expression were reduced (SePP, pGPx, plasma Se, Dio2 activity in fibroblasts), alterations of thyroid hormone metabolism were the only phenotypes reported (28). In particular, the apparent pituitary resistance to T4 was compatible with a reduced expression of Dio2 in this tissue and in line with the results from the *Dio2*-deficient mice.

Muscle

The first genetic disease involving a selenoprotein was rigid-spine muscular dystrophy and Mallory body disease (63). Mutations in the human gene encoding SelN, *SEPN1,* affect either the coding sequence or the SECIS element, pointing to an essential role of Sec in SelN function. In zebrafish, it was shown that knockdown of SelN leads to a loss of axial muscle structure (25). Recently, a molecular and functional association of SelN with the ryanodin receptor has been demonstrated, thus providing for a functional role for this selenoprotein (55). Whether lack of SelN or lack of another selenoprotein (or selenoproteins) underlies (underlie) "white muscle disease" of calves fed a Se-deficient diet has not been resolved. Interestingly, muscle regeneration seems to be augmented in a mouse model with increased expression of some and decreased expression of other selenoproteins due to a mutant $tRNA^{[Ser]Sec}$ transgene (51).

Testis and Male Fertility

For >4 decades, Se has been recognized as an essential factor for male fertility (reviewed in (33)). Rodents kept for several generations on a Se-deficient diet develop male infertility, evidenced by severe morphological aberrations of isolated sperm, such as giant heads, breaks and bends in the midpiece of sperm, and protrusion of outer dense fibers and testicular atrophy.

Inactivation of selenoprotein P

Genetic disruption of the selenium transport protein SePP causes male infertility with kinked sperm (71, 77). The sperm phenotype and fertility in *Sepp*-deficient mice were rescued by hepatic expression of SePP, demonstrating that SePP is the carrier delivering hepatic Se to the testis (77). While provision of inorganic Se (selenite) can normalize (*e.g.*, kidney Se levels), selenite is not able to cross the blood-testis barrier. There, ApoER2 is expressed and mediates receptor-dependent endocytosis of SePP (70). These studies, however, did not clearly assign a molecular function for a specific selenoenzyme in male fertility.

Glutathione peroxidase 4 (GPx4)

Due to its markedly high expression in testis, GPx4 has been long suspected to confer an important function in male fertility. However, it was subsequently shown that several other selenoproteins, such as TGR and SelV, occurred in testis and that their expression was quite restricted in this tissue (57).

A major breakthrough was made in 1999 when Ursini and colleagues were searching for a selenoprotein whose function must be linked to the severe sperm abnormalities manifested primarily in the midpiece. For many years, the sperm mitochondria-associated cysteine-rich protein (SMCP) was considered to be a 20 kDa selenoprotein and the predominant capsular protein. SMCP is confined to the midpiece of spermatozoa, consists of ~18% of cysteine, and has the essential in-frame UGA codon. For these reasons, SMCP was long assumed to be the major target of selenium-deficiency. However, several studies failed to show that this protein in fact contains covalently bound Se. Surprisingly, GPx4 was identified as the major structural component (up to 50%) of the mitochondrial capsules which embed sperm mitochondria and are required for stability of the spermatozoan midpiece (105). Therein, GPx4 was found to be cross-linked to other capsular proteins via (selenenyl-)disulfide bridges. This is due to a unique catalytic feature of GPx4, which under conditions of GSH deprivation—as physiologically evident in maturing sperm—uses thiol groups from neighboring proteins, thus introducing disulfide bridges into proteins (62).

As three different forms of GPx4 with different subcellular localization, a cytosolic (cGPx4), a mitochondrial (mGPx4), and, as subsequently shown, a nuclear (nGPx4) arise from one gene (72), it was not clear at that time if one or all of them are indeed required for male fertility. Furthermore, studies with constitutive inactivation of the entire *Gpx4* gene could not provide any evidence as knockout mice die during early embryonic development (115). Initial studies by Pushpa–Repka and colleagues suggested that the mitochondrial variant is almost exclusively expressed in testis (74), and as later shown by expression analysis thus displays a similar expression signature as the nuclear transcript (81). From these findings, it was hypothesized that both isoforms might be dispensable for embryonic development.

Inactivation of the nuclear form of GPx4

Mice were generated which specifically lack expression of the nuclear variant (19). This approach was easily performed, as the nuclear variant is expressed from its own promoter and differs from the two other forms by an N-terminal extension. Mice with specific disruption of the nuclear form were fully viable and virtually normal. Surprisingly, nGPx4 null males

were fully fertile, and testicular tissue and isolated spermatozoa did not show any gross morphological abnormalities, raising the question why sperm cells express a selenoenzyme that is apparently dispensable for male fertility. Despite this rather unexpected finding, it was asked whether nGPx4 may act as a protein thiol peroxidase *in vivo*. Studies with isolated sperm in fact confirmed that the oxidation of sperm protamines was clearly reduced in knockout animals (19). Yet, this study was not affirmative for the proposed essential function of GPx4 in mammalian fertility.

Mitochondrial form of GPx4

The next logical step was to specifically destroy the mitochondrial variant (80). This was, however, far more difficult than targeting the nuclear form, because the transcription initiation at the first exon determines if the longer (mitochondrial) or the shorter (cytosolic) form is generated. To this end, an in-frame stop codon was inserted amid the mitochondrial and the cytosolic ATGs, leading to disruption of the mGPx4 with little or no impact on the expression of cGPx4. Germ line transmission of the targeted allele was achieved only via female foster mice, which indicated haploinsufficiency.

Like nGPx4-deficient mice (19), mGPx4 null mice were born at the Mendelian ratio and were shown to be fully viable (80). Expression analysis of different adult and embryonic tissues revealed that there were no differences in GPx4 expression, except testis where this selenoenzyme was found to be strongly diminished. These studies showed for the first time that the mitochondrial variant is the prevailing form in testis. The equal size of the cytosolic and mitochondrial forms, after post-translational processing of the mitochondrial targeting signal, had precluded showing this previously. It was also demonstrated that the mitochondrial form is located predominantly in the sperm midpiece; other parts of the sperm, including head and acrosome and tail, were spared by the knockout.

The major finding of this study (80), however, was that male knockout mice are infertile as proven by test breeding with wild-type females. Also, isolated sperm failed to fertilize oocytes *in vitro*, most likely due to the severe impairment in sperm motility and progressivity. Interestingly, the inability of knockout sperm to generate viable offspring could be bypassed by injecting sperm heads directly into oocytes, which might be of clinical relevance. Ultrastructural analysis revealed severe sperm abnormalities in the midpiece of mature spermatozoa, which recapitulated features typical of severe seleno-deficiency in rodents as described much earlier (109, 110). mGPx4 is thus required as an essential structural component of mitochondrial capsule, but it is also involved in the cross-linking of capsular proteins, as proposed previously by Ursini's laboratory (61).

These studies firmly establish that mGPx4 confers the vital role of selenium in male fertility (80), an observation which had been made >40 years ago (45). On the other hand, the investigations with the mGPx4 knockout mice and cells did not support the previously drawn conclusion (mainly obtained by cell culture studies) (80), where an essential role in apoptosis regulation was postulated for mGPx4 (see below). Interestingly, mice overexpressing mGPx4 from the mouse synaptonemal complex protein 1 promoter in mice were also shown to have reduced male fertility due to increased apoptosis of primary spermatocytes, loss of haploid cell, and a disorganized seminiferous epithelium (73).

Other selenoproteins

The investigations with the nGPx4 and mGPx4 null mice imply that Cre/loxP-mediated disruption of the entire *Gpx4* gene in somatic tissues will unmask cGPx4 functions (see below). On the other hand, as some phenotypes such as testicular atrophy could not be observed in the knockout mice, it must be concluded that one or several selenoproteins other than mGPx4 contribute to testis physiology. For instance, cGPx4 might be important for proper sperm development by protecting germ cells from oxidative stress, as previously described for neurons (94). In addition, SelV with yet-unknown function also shows a testis-specific expression pattern (57) and may have a role in this process. TGR has been considered to act hand in hand with nGPx4 in introducing and reshuffling proper disulfide bridges into proteins including protamines (102).

Future studies with knockout mice for selenoproteins will certainly unmask their physiological significance and may provide novel molecular functions in sperm maturations. Yet it remains to be shown that genetic mutations in one or several testis-specific selenoproteins may impact on male fertility. Even though polymorphisms have been described for the human *GPX4* gene, a clear correlation could not be provided between GPx4 mutations and male fertility (60).

Brain

Inactivation of selenoprotein P

Feeding experimental animals Se-deficient diets over many generations did not generate overt neurological deficits. This finding may relate to the preservation of brain Se content during dietary Se restriction (3). Thus, the first animal model in which brain Se content was significantly reduced was the *Sepp*-deficient mouse (48, 86). These mice suffer from spontaneous neurological deficits including ataxia and seizures, both of which were responsive to inorganic Se supplementation (49, 89). Interestingly, only relatively unspecific histopathological findings have been described in *Sepp*$^{-/-}$ mice, including brain stem axonal degeneration and astrogliosis (107). The identification of ApoER2 as a SePP receptor in testis (70) paved the way for its identification as a SePP receptor in brain (12). Again, *ApoER2*-deficient mice suffered the same histopathology as *Sepp*-deficient mice when fed a Se-deficient diet (12). These and other studies (76, 92) suggest that neurons are privileged targets for SePP by their expression of ApoER2.

Abrogation of selenoprotein biosynthesis

While these studies unequivocally showed that Se is important for the brain, they did not specify if selenoproteins are the mediators and which selenoprotein might be essential in brain function. Clearly, the supposed involvement of oxidative stress in neurodegenerative disease suggested such a link (87). We have approached this question by generating mice

deficient in all neuronal selenoproteins by conditional inactivation of $tRNA^{[Ser]Sec}$. The devastating neurological phenotype of neuron-specific selenoprotein knockout animals strongly supported a role of selenoproteins for neuronal survival and brain development (91). While the neurobiological studies into mechanisms are still underway, seizures and cerebellar defects support the notion that cerebral selenoprotein deficiency may be the reason for the neurological phenotype of *Sepp*$^{-/-}$ and *ApoER2*$^{-/-}$ mice.

Thioredoxin/thioredoxin reductase system

Transgenic overexpression of thioredoxin was shown to protect brain from various inducers of oxidative stress, including focal brain ischemia induced by middle cerebral artery occlusion, kainic acid-induced hippocampal damage and seizures, and mild focal ischemia. Conversely, *Trx2*-deficient embryos die around mid-gestation because of widespread apoptosis, including in brain (68). As thioredoxin activities are controlled by thioredoxin reductases, it was interesting to assess whether loss of one of the thioredoxin reductase(s) may provoke a phenotype in brain. Interestingly, brain-specific deletion of Txnrd2 did not have any impact on the developing brain, nor did it trigger neurodegeneration (99). This was somewhat surprising as mitochondrial dysfunction has been often linked to neurodegenerative disease (58), and *Trx2* knockout embryos were shown to have increased numbers of TUNEL-positive cells in brain (68).

By stark contrast, Txnrd1 is essential for proper cerebellar development (99). Mice with brain-specific inactivation of *Txnrd1* were born at the Mendelian ratio. However, *Txnrd1* knockout mice were reduced in body size and showed ataxia (99). As the cerebellum is required for the coordinated movement, it was not surprising that major defects were observed in the cerebellum, which mainly develops in the first 3 weeks after birth in mice. Progressive histopathological abnormalities include a reduced thickness of the external granular layer, which is the origin of cells for the granular layer, the absence of lobules V to I in the anterior region, and ectopically localized Purkinje cells with aberrant dendritic arborization. A sharply reduced number of proliferating cells in the granular layer of the anterior cerebellum was demonstrated to be the underlying reason for the striking cerebellar hypoplasia, which perfectly fits with the severe proliferation defects of *Txnrd1* knockout embryos (54). Neuron-specific ablation of *Txnrd1* did not cause any of the aforementioned aberrations indicating that non-neuronal cells depend on functional Txnrd1 (99). This is substantiated by the notion that the Bergmann glial cells, which are essential for neuronal migration during cerebellar development, were disoriented, shortened and reduced in density.

Glutathione peroxidase system

Neuronal glutathione (GSH) deficiency has been linked to neurodegenerative disease, and mice lacking excitatory amino acid carrier-1, a glutamate and cysteine transporter, develop brain atrophy and behavioral changes (1). As γ-glutamyl-cysteine-synthetase knockout mice die during early embryonic development just after gastrulation (E7.5) (96), no other independent information on the role of genetic GSH-deficiency in the brain had been available. However, recent studies with *Gpx1* knockout mice and neuron-specific *Gpx4* knockout mice provided evidence that glutathione peroxidases in fact confer crucial functions in brain.

GPx1, the first mammalian selenoprotein to be discovered (34, 78), was targeted for removal in mice as early as 1997 (50). As GPx1 was considered to be the prototype Se-dependent glutathione peroxidase, it was considered to be of vital importance for many physiological functions. But knockout studies in mice disproved that GPx1 is a highly significant selenoenzyme (50). *Gpx1*$^{-/-}$ mice are fully viable and only develop phenotypes when challenged with by hypoxia or neurotoxins (reviewed in (90)). For instance, GPx1 null mice have a much greater susceptibility to cerebral injury following focal ischemia, which was eventually reasoned that GPx1 is normally required to maintain microvascular perfusion (113). The same group also showed that GPx1-deficient neurons were less protected from ß-amyloid-induced toxicity (21). On the contrary, *Gpx1* transgenic mice and isolated primary hippocampal neurons were surprisingly found to be less resistant to the neurotoxin, kainic acid, used to induce seizures. The authors speculated that this may result from an increased activation of NMDA receptors or downstream signaling due to altered intracellular redox balance (8).

While these studies pointed to an important function of GPx1 in protecting the brain from oxidative insults, the physiological role of GPx4 has been less understood, which was certainly due to the lack of an appropriate genetic model for a long time. GPx4 is fairly highly expressed in brain and its expression is strongly maintained even under prolonged Se-deficiency, indicating that this selenoprotein may play a crucial role in brain tissue (85). Consequently, gain-of function studies in mice showed that primary culture cortical neurons overexpressing GPx4 are protected from exogenous oxidative stress-inducing agents, like hydrogen peroxide and *t*-butyl hydroperoxide, in addition to ß-amyloid-induced toxicity (reviewed in (17)).

To further analyze the importance of GPx4 in neuronal cells, GPx4 was deleted in functional neurons using mice with loxP-flanked *Gpx4* alleles and the *CamKIIα-Cre* transgenic mice (94). Soon after birth, neuron-specific GPx4 null mice developed an atactic gait, were hyperexcitable, and apparently suffered seizures when touched. The mice thus resembled *Sepp*-deficient mice fed a low Se diet.

To study the molecular mechanisms of GPx4 in redox-regulated cell death signaling pathways, *Gpx4*$^{-/-}$ mouse embryonic fibroblast (MEFs) were generated from the conditional *Gpx4* knockout embryos (94). Inducible GPx4 deletion was shown to cause rapid cell death due to massive lipid peroxidation, which could be prevented by low micromolar concentrations of the lipophilic antioxidant α-tocopherol, but not by water-soluble antioxidants. This finding was not surprising as GPx4 was discovered as an enzyme that efficiently protects biomembranes from peroxidative degradation (106). Pharmacological studies subsequently revealed that 12/15-lipoxygenase (12/15-LOX) specifically sparks lipid peroxidation and cell death in *Gpx4*$^{-/-}$ cells (94). The fact that lipid peroxidation is specifically generated by an enzyme in cells and not secondary to other reactions, however, was in fact surprising. In this context, it is noteworthy that targeted deletion of 12/15-LOX in mice protects from stroke in a manner similar to pharmacological inhibition of 12/15-LOX in

wild-type mice (108). Activation of apoptosis-inducing-factor (AIF), a pro-apoptotic enzyme in mitochondria, was further identified as an effective executor of cell death, which occurred independently of caspase activation (94). As the identified pathway was shown to act also in neurons (see above), one can conclude that this novel oxidative stress-induced signaling pathway is of paramount importance for neurons (94). On the other hand, the nuclear and mitochondrial variants of GPx4 did not emerge to play a substantial (protective) role in brain development and apoptosis regulation, as shown by individual disruption of both forms in mice (19, 80). This was somewhat surprising as numerous overexpressing studies using mGPx4 or RNAi-mediated knockdown of mGPx4 pointed to an important role in brain development and protection from oxidative stress (reviewed in (17) and (20)).

Methionine sulfoxide reductases

Targeted deletion of the non-Se methionine-S-sulfoxide reductase (MsrA) in mice led to "tip-toe walking" and protein oxidation, which was exacerbated by Se deficiency (65). MsrB1 reduces the R-isomer of MetSO and is a selenoenzyme, SelR. Targeted inactivation of SelR, however, did not produce a neurological deficit (35), albeit these mice were not kept on a Se-deficient diet. Although reversible regulation of ion channels and signaling molecules by methionine oxidation represents an attractive hypothesis how Se (via MrsB1) could impact on neuronal signaling, at present there are no data to specifically support this idea.

Hypomorphic tRNA$^{[Ser]Sec}$

Expressing a hypomorphic allele of tRNA$^{[Ser]Sec}$ in a transgenic mouse model (*Trsp*$^{\Delta AE}$) led to a general reduction of selenoprotein expression, including in brain (13). These mice strikingly resemble *Sepp*$^{-/-}$ mice in several aspects, including the seizure and movement phenotype. Histologically, we found general astrogliosis and a notable reduction of cerebral inhibitory interneurons immuno-positive for the marker protein parvalbumin. This finding is well in line with the seizure phenotype of the mice and represents the first specific histopathological phenotype of selenoprotein-deficient mice. Interestingly, we had also found a specific loss of parvalbumin-positive hippocampal and cortical interneurons in neuron-specific *Gpx4*$^{-/-}$ mice (94). *Trsp*$^{\Delta AE}$ mice also have reduced cerebral GPx4 protein expression (like *Sepp*$^{-/-}$ mice), a feature that seems specific to all selenoprotein-deficient mouse models that have spontaneous neurological phenotypes.

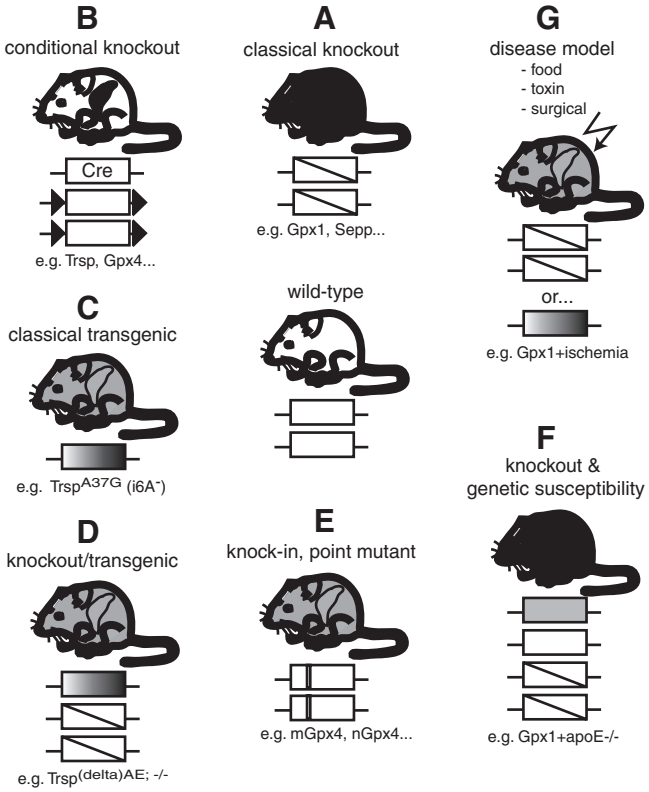

FIG. 2. Experimental approaches for the targeted genetic manipulation of (seleno)proteins in mice. (A) Classical gene targeting ("knockout") usually leads to systemic inactivation of gene function. The advantage, a 100% loss-of-function, is also the disadvantage of the method, as in humans mild genetic variations are more widespread than complete inactivation. (**B**) Conditional (*i.e.* cell type-specific or inducible). Cre recombinase-mediated gene inactivation offers the advantage to (i) bypass possible embryonic lethality and (ii) to study the role of an individual protein in a given organ or cell type *in vivo* or *ex vivo*. Transgenic expression of a variant target gene without (**C**) or with (**D**) concomitant gene targeting of the wild-type allele offers the advantage to study dominant effects or to complement the loss of wild-type protein. (**E**) Specific engineering of the endogenous allele ("knock-in" or targeting of alternative exons, *i.e.*, isoforms) allows for manipulations more closely resembling the situation in humans. Finally, all these genetic manipulations of target genes can be combined with either another genetic disease model (**F**) or with dietary, pharmacological/toxicological, or surgical interventions (**G**) representing models for human diseases.

Outlook

The application of reverse mouse genetics has immensely promoted the elucidation of the physiological functions of Se-dependent proteins. Classical and conditional deficiency models and (tissue-specific) overexpression of wild-type or mutant genes are now standard technology, and in short time all selenoprotein genes will have been inactivated in the mouse (Fig. 2). This will, however, only provide the basis for studies into the physiological function and possible modifications of (genetic) disease. The next step will be to cross-breed selenoprotein-deficient mice with models of genetic disease (*e.g.*, apoE- or cystathione-β-synthase-deficient mice) or to subject genetically modified mice to pharmacological or experimental models of human disease. Given the increasing evidence for modulation of cancer incidence by single-nucleotide-polymorphisms in selenoprotein genes, the future may see transgenic mouse models with incomplete disruption of selenoprotein function. Although the many health claims for Se are sometimes questioned, several key findings from nutritional experiments are now being supported by more direct molecular transgenic studies.

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Abbreviations Used

AIF = apoptosis inducing factor
apoE = apolipoprotein E
CFS = cerebrospinal fluid
cGPx4 = cytosolic GPx4
Dio = deiodinase
E = embryonic day
FAP = familial adenomatous polyposis coli
GPx = glutathione peroxidase
GSH = glutathione
12/15-LOX = 12/15-lipoxygenase
MEFs = mouse embryonic fibroblasts
mGPx4 = mitochondrial GPx4
MsrA = methionine-S-sulfoxide reductase
nGPx4 = nuclear GPx4
NO = nitric oxide
Sec = selenocysteine
SECIS = selenocysteine insertion sequence
SECISBP2 = SECIS-binding protein 2
SelN = selenoprotein N
Sepp = selenoprotein P
SMCP = sperm mitochondria-associated cysteine-rich protein
SPS2 = selenophosphate synthetase
T3 = 3,3′,5-triiodothyronine
T4 = thyroxine
TGR = thioredoxin glutathione reductase
TRH = thyrotropin-releasing-hormone
Trx = thioredoxin
TSH = thyroid-stimulating-hormone
TUNEL = TdT-mediated dUTP-biotin nick end labeling
Txnrd = thioredoxin reductase
3′-UTR = 3′-untranslated region

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