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Chapter 29. Mouse models for assessing the role of selenoproteins in health and development

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Summary: Mouse models have been generated to assess the roles of selenoproteins involved with housekeeping tasks and/or stress-related phenomena in development and health. Each mouse model has taken advantage of the fact that the synthesis of all selenoproteins is dependent on the expression of two selenocysteine (Sec) tRNA$^{[Ser]Sec}$ isoforms that differ from each other by a single methyl group on the ribosyl moiety at position 34. The endogenous Sec tRNA$^{[Ser]Ssc}$ population was selectively altered by generating mouse models involving 1) transgenic animals carrying mutant or wild type Sec tRNA$^{[Ser]Sec}$ transgenes, 2) conditional knockout animals carrying a floxed Sec tRNA$^{[Ser]Sec}$ gene that was targeted for removal in specific tissues and organs using *loxP-Cre* technology and 3) transgenic/standard knockout animals carrying mutant or wild type transgenes and a knockout of the Sec tRNA$^{[Ser]Sec}$ gene wherein the animal's survival is dependent on the transgene. These mouse models perturbed selenoprotein expression, often in a protein- and tissue-specific manner, permitting us to better assess their function in health and development.

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Introduction

Numerous human clinical trials examining the role of selenium in health have been initiated recently, or have already been completed. Perhaps the most famous of those studies that have been completed is that of Clark et al [1]. These investigators reported in 1996 that supplementing the diet with 200 micrograms of selenium/day reduced the incidence of prostate and colon cancers by more than 50% and lung cancer by about 35%. This study provided the basis for numerous subsequent human clinical trials involving selenium. For example, the largest human clinical trial ever undertaken, the Selenium and Vitamin E Cancer Prevention Trial (**SELECT** [2]), involves more than 35,000 males in determining whether selenium and vitamin E may play a role in protecting men against prostate cancer. Another human clinical trial recently undertaken involves 1960 individuals in determining whether selenium may have a role in preventing the occurrence of secondary lung tumors in patients with a previous surgically removed non-small cell lung lesion [3]. These trials are being carried out at the cost of hundreds of millions of dollars with little or no understanding of how selenium acts metabolically to bring about potential health benefits. It is important therefore to generate mouse models to determine how selenium acts at the molecular level in promoting better health and to determine whether small molecular weight selenocompounds and/or selenoproteins are the responsible agents.

Selenoproteins are dependent on Sec tRNA$^{[Ser]Sec}$ for their expression as discussed in detail in Chapter 3 (see also [4,5]). In addition, the Sec tRNA$^{[Ser]Sec}$ population in mammalian cells is composed of two major isoforms that differ from each other by a 2'-*O*-methylribosyl moiety at position 34 which is designated Um34 (Chapter 3 and [4,5]). The two isoforms, designated mcm^{5}U and mcm^{5}Um, have different roles in selenoprotein synthesis wherein mcm^{5}U appears to be largely responsible for the expression of housekeeping selenoproteins and mcm^{5}Um largely responsible for selenoproteins involved in stress-related phenomena [6,7].

By perturbing the expression of Sec tRNA$^{[Ser]Sec}$ in mice, selenoprotein synthesis can be altered in such a way that their roles in development and health can be readily studied. In fact, Sec tRNA$^{[Ser]Sec}$ synthesis can be modulated in various ways in generating different mouse lines so that the roles of selenoproteins involved in housekeeping tasks and in stress-related phenomena can be elucidated. We have generated several mouse models for examining the role of selenoproteins in development and health. These mouse models involve generating transgenic, standard knockout, conditional knockout or a combination of transgenic/knockout mice encoding either wild type or a mutant Sec tRNA$^{[Ser]Sec}$ gene or transgene. It should also be noted that the characteristics and properties of a number of individual selenoproteins have been examined, including being targeted for removal, to

assess their roles in health and development (see the Chapters in Part II. Selenium-containing proteins).

Mouse models for elucidating the role of selenoproteins in development and health

To generate mouse models for elucidating selenoprotein function, we took advantage of the fact that selenoprotein synthesis is dependent on the presence of Sec tRNA[Ser]Sec. Our mouse models involve the introduction of a transgene, designated *trsp[t]*, or manipulation of the Sec tRNA[Ser]Sec gene, designated *trsp* and they are summarized as follows: 1) transgenic mice carrying wild type or a mutant *trsp[t]* [8]; 2) conditional knockout mice carrying a floxed *trsp* that can be specifically targeted for removal using *loxP-Cre* technology [9]; and 3) standard knockout/transgenic mice containing *trsp* knockout, designated Δ*trsp*, and carrying wild type or mutant *trsp* transgenes in which the survival of the animal is dependent on the transgene [6,7]. A fourth mouse model is also briefly discussed wherein transgenic/conditional knockout mice carrying two transgenes, a *Cre* transgene under control of a promoter targeted for a specific organ or tissue, and a wild type or mutant *trsp[t]* in addition to the targeted (floxed) *trsp* (B.A. Carlson, M.E. Moustafa, R. Shrimali, M. Rao, N. Zhong, S. Wang, L. Feigenbaum, B.J. Lee, V.N. Gladyshev and D.L. Hatfield, submitted). The mouse models are shown in Table 1. They were prepared for assessing the roles of selenoproteins in health and development as well as defining the roles of the two Sec tRNA[Ser]Sec isoforms, mcm^{5}U and mcm^{5}Um, in selenoprotein synthesis. Since the role of mcm^{5}U, which is largely involved in the expression of housekeeping selenoproteins, and of mcm^{5}Um, which is largely involved in the expression of selenoproteins involved in stress-related phenomena, has been discussed in Chapter 3 and detailed elsewhere [5-7], the emphasis of the present chapter is on generating mouse models for examining the roles of selenoproteins in health and development. It should be noted that some of our mouse models may also be used to elucidate the role of small molecular weight selenocompounds in disease prevention.

Transgenic mouse models

Transgenic mice are generated by introducing one or more copies of the wild type or mutant *trsp* into the mouse genome. The resulting mice are then bred to obtain a stable breeding population (i.e., mice that are homozygous for the transgene). Mutations were made at either position 37 [8] or at postion 34 in Sec tRNA[Ser]Sec for preparing mutant transgenes. The base at position 37 in the fully maturated tRNA[Ser]Sec is isopentenyladenosine (i^{6}A), while that at position 34 is mcm^{5}U (see Chapter 3 or [4,5]). The base at position 37 was changed to G (A37->G37) and that at position 34 to A (T34->A34). Importantly, neither of these two, very different, mutant tRNAs contain

Um34, the methyl group located at the 2'-hydroxylribose on mcm⁵U [10]. An A in the wobble position of the anticodon in any tRNA is normally converted to I. About 65% of the A34 mutant Sec tRNA[Ser]Sec is converted to I34 in mouse liver (B.A. Carlson, M.E. Moustafa, R. Shrimali, M. Rao, N. Zhong, S. Wang, L. Feigenbaum, B.J. Lee, V.N. Gladyshev and D.L. Hatfield, submitted); and interestingly, the ACA and ICA anticodons in A34 Sec tRNA[Ser]Sec decode UGU and UGU, UGC (UGU and UGC are codons for cysteine) and UGA (UGA is the codon for Sec), respectively.

Table 1. Mouse models and their uses.

Type	Genotype[a]	Uses[b]
Transgenic	$trsp^t$	Determine if Sec tRNA[Ser]Sec is limiting [8] SP[c] rescue in standard KO[d] [6,7] SP[c] replacement in targeted tissues and organs[e]
Transgenic	$G37trsp^t$	Rescue of SPs in KO[d] mice Replacement of SPs in conditional KO[d] mice Roles in muscle adaptation [9] and cancer risk in prostate,[e] mammary[e] gland and colon[f]
Transgenic	$A34trsp^t$	SP[c] replacement only
Standard KO[d]	$\Delta trsp$	SP[c] rescue with $trsp^t$ & $trsp^t$G37
Conditional KO[d]	$\Delta trsp^f$	KO[d] trsp in various tissues and organs using promoters that are tissue and organ specific
Transgenic/ standard KO[d]	$trsp^t/\Delta trsp$ $G37trsp^t/\Delta trsp$	SP[c] rescue in KO[d] mice [6,7] Partial SP[c] rescue in KO[d] mice [6,7]
Transgenic/ conditional KO[d] (liver)	$trsp^t/\Delta trsp^{fl}$ $G37trsp^t/\Delta trsp^{fl}$	SP[c] replacement in liver[g] SP[c] replacement in liver[g]

[a]Genotype designations used for mouse models (see text).
[b]Uses – the various uses of the mouse models with accompanying references (see also text).
[c]SP – selenoprotein(s).
[d]KO – knockout.
[e]AM Diamond, personal communication.
[f]R Irons, BA Carlson, DL Hatfield, C Davis, submitted.
[g]BA Carlson, ME Moustafa, R Shrimali, M Rao, N Zhong, S Wang, L Feigenbaum, BJ Lee, VN Gladyshev and DL Hatfield, submitted.

Initially, the resulting transgenic mice encoding multiple copies of *trsp^t* and G37*trsp^t* (see Table 1) were examined [8]. This study provided the first example of transgenic mice engineered to contain functional tRNA transgenes. The fact that over-expression of wild type Sec tRNA[Ser]Sec due to the extra copies of *trsp^t* did not appear to influence selenoprotein synthesis in the organs and tissues examined suggested that the levels of the Sec tRNA[Ser]Sec isoforms were not limiting in protein synthesis (reviewed in [4,5]). However, mice carrying G37*trsp^t* had a pronounced effect on selenoprotein expression and the effect occurred in a protein- and tissue-specific manner [8]. The most and least affected selenoproteins were glutathione peroxidase 1 (GPx1) and thioredoxin reductase 1 (TR1), respectively, and the organs which manifested the most and least affect on selenoprotein synthesis were liver and testes, respectively. The mutant Sec tRNA[Ser]Sec product from G37*trsp^t* lacked the i^6A base modification and altered the levels of the two host Sec tRNA[Ser]Sec isoforms is such a manner that the Um34 species was reduced and the mcm^5U species was enriched. As the amount of the mutant tRNA increased with increasing numbers of transgenes, the amount of the Um34 species and the amount of some selenoproteins, and in particular, GPx1 also decreased. The correlation in reduction of the Um34 isoform and certain selenoproteins led us to propose that the Um34 modification is responsible for the expression of several selenoproteins that are involved in the lower echelon of selenoprotein hierarchy expression [5-7]. Interestingly, many of the selenoproteins that are expressed in the lower echelon of selenoprotein hierarchy and are sensitive to selenium status, such as GPx1, serve largely stress-related functions, while those that are expressed in the upper echelon of selenoprotein hierarchy and are less sensitive to selenium status, such as TR1, serve largely housekeeping functions. Those members associated with stress-related phenomena are the ones dependent on the Sec tRNA[Ser]Sec Um34 modification for their expression (see also Chapter 3 and [5-7]).

As shown in Table 1, the G37*trsp^t* mice have been used in several different studies. The studies have shown that these mice, which are deficient in selenoproteins involved in stress-related phenomena, have an enhanced 1) skeletal muscle adaptation after synergist ablation and following exercise [9], 2) incidence of prostate malignancy when the mice also carry an oncogene directed to this tissue (see legend to Table 1), and 3) incidence of breast malignancy when the mice also carry an oncogene directed to this tissue (see legend to Table 1). In addition, we have observed that G37*trsp^t* mice a significantly greater number of azoxymethane (AOM)-induced aberrant crypt formations (preneoplastic lesions in the colon) than wild type mice (R Irons, BA Carlson, DL Hatfield and CD Davis, submitted). Supplementing the diets of the AOM treated G37*trsp^t* and wild type mice with 0.1 and 2.0 µg/g selenium significantly reduced the incidence of preneoplastic lesions in both

mouse lines. These observations are the first to provide evidence that both selenoproteins and low molecular weight selenocompounds play a role in the cancer protective effects of selenium.

In another study involving Sec tRNA[Ser]Sec mutant transgenic mice, we examined the effect of A34*trsp*[t], which also lacked Um34, on selenoprotein synthesis (B.A. Carlson, M.E. Moustafa, R. Shrimali, M. Rao, N. Zhong, S. Wang, L. Feigenbaum, B.J. Lee, V.N. Gladyshev and D.L. Hatfield, submitted). The effects of A34*trsp*[t] on down regulating stress-related selenoprotein expression were similar to those of G37*trsp*[t]. Since both these mutants lack Um34, these observations provided further evidence that Um34 is responsible governing the expression of those selenoproteins involved in stress-related phenomena.

Transgenic/knockout mouse models

Since the standard knockout of *trsp* is embryonic lethal [9,11], it appeared that this mutant could not be used for further study of selenoprotein expression. However, we devised a means of rescuing selenoprotein expression by crossing heterozygous *trsp* knockout mice with homozygous *trsp*[t] transgenic mice and breeding the offspring to obtain a line of mice lacking *trsp* (Δ*trsp*) that was dependent on the *trsp*[t] for survival [6,7]. One advantage of rescuing a knockout mouse with a wild type or mutant transgene is that the number of transgenes, and therefore, the levels of the corresponding gene product can be maintained at normal or elevated amounts depending on the transgene copy number. Rescuing with 20 copies of the wild type transgene enriched the Sec tRNA[Ser]Sec population several fold, but little or no effect on selenoprotein expression in various tissues or organs was observed [6,7]. These observations provided further evidence that Sec tRNA[Ser]Sec is not limiting in selenoprotein biosynthesis (reviewed in [4,5]). Rescue of the Δ*trsp* mice with the mutant transgene, G37trsp[t] afforded us with an opportunity of obtaining a mouse line with a mutant transgene wherein there is no background of host Sec tRNA[Ser]Sec and selenoprotein expression is therefore totally dependent on the mutant tRNA. G37*trsp* yielded a tRNA that lacked two base modifications, i[6]A37 and Um34 (see above, Chapter 3 and [6,10]) and mice rescued with G37*trsp*[t] lacked several selenoproteins including glutathione peroxidases 1 and 3, SelR and SelT [6,7]. Interestingly, we were not successful in rescuing selenoprotein synthesis in Δ*trsp* mice with A34*trsp*[t] (see reference to Carlson et al above). As discussed in greater detail in Chapter 3, the novel regulation of several selenoproteins involved in stress-related functions occurs at the level of translation.

Conditional knockout mouse models

Removal of *trsp* from the mouse genome is embryonic lethal [9,11] which prevented further study of the standard *trsp* knockout *per se* in selenoprotein expression. We therefore prepared the conditional knockout of *trsp* using *loxP-Cre* technology [9]. The removal of floxed *trsp* in mouse mammary epithelium [9] and in liver hepatocytes [12] was examined. *trsp* was targeted for removal in mammary epithelium using transgenic mice carrying the Cre recombinase gene under the control of the mouse mammary tumor virus long terminal repeat promoter or the whey acidic protein promoter. Neither *Cre* promoter was effective in complete removal of *trsp* in mammary epithelial cells, but the Sec tRNA$^{[Ser]Sec}$ population was substantially reduced to alter selenoprotein expression in a protein specific manner [9]. In liver, however, the targeted removal of floxed *trsp* with transgenic mice carrying the Cre recombinase under the control of the albumin promoter was virtually complete [12]. Surprisingly, the mice survived without selenoprotein expression in hepatocytes which comprise about 85% of the liver cell mass. Selenoprotein P (SelP), which is the only known selenoprotein with multiple Sec residues (see Chapters 9, 10 and 21), would seem to be largely made in the liver and transported to other organs and tissues as its level was reduced about 75% in plasma of the selenoproteinless liver knockout mice. These mice appeared phenotypically normal until about 24 hours before death and death appeared to be due to severe hepatocellular degeneration and necrosis with concomitant necrosis of peritoneal and retroperitoneal fat [52]. Although most animals lacking selenoprotein expression in their liver died within two to three months in this initial study, these animals may be kept alive for extended periods of time on a diet enriched in other nutrients (U. Schweizer, L. Schomburg and J. Kohrle, personal communication). This is an important observation since these animals live much longer on a different diet and can be subjected to various environmental agents to study the role of selenoproteins in liver function and health.

As selenium has been implicated in heart disease (see Chapter 25) and immune function (Chapter 27), we examined the role of selenoproteins in cardiovascular disease and the immune system. By targeting the removal of *trsp* in either endothelial cells or myocytes in skeletal and heart muscle, we have elucidated the role of selenoproteins in cardiovascular disease (R. Shrimali, J.A. Weaver, G.R. Miller, B.A. Carlson, S.V. Novoselov, E. Kumaraswamy, V.N. Gladyshev and D.L. Hatfield, submitted). Removal of selenoprotein expression in endothelial cells was embryonic lethal. 14.5-day-old embryos had numerous abnormalities including necrosis of the central nervous system, subcutaneous hemorrhage and erythrocyte immaturity. Loss of selenoprotein expression in myocytes, however, manifested no apparent phenotype until about day 12 after birth, when affected mice developed decreased mobility and an increased respiratory rate, followed by death

within a few hours. Although there was no evidence of inflammation of the skeletal muscle in mice that lacked *trsp* in myocytes, they had moderate to severe myocarditis with inflammation extending into the mediastinum.. Targeted removal of *trsp* in endothelial cells demonstrated an essential role of selenoproteins in their development, while targeted removal of *trsp* in myocytes demonstrated an essential role of selenoproteins in proper function of cardiac muscle. These studies also showed a direct connection between the loss of selenoprotein expression and cardiovascular disease (R. Shrimali, J.A. Weaver, G.R. Miller, B.A. Carlson, S.V. Novoselov, E. Kumaraswamy, V.N. Gladyshev and D.L. Hatfield, submitted).

The conditional knockout of *trsp* is an important mouse model as it provides a means of elucidating the roles of selenoproteins in health, development and/or function in tissues or organs for which there is a specific promoter. Promoters that function to express Cre early in development of a specific tissue or organ can be used to assess the role of selenoproteins in development of that tissue or organ. On the other hand, promoters that function to express Cre after the tissue or organ is developed can be used to assess the role of selenoproteins in proper function of that organ or tissue. Furthermore, promoters that function to express Cre either early or late in development of a specific tissue or organ, and provided the animal survives for a long period of time following *trsp* knockout, can be used to examine the animal's ability to handle various forms of stress (e.g., viral or bacterial infection, carcinogen(s), cancer driver gene(s), etc). Those animals that survive for long periods of time, while lacking selenoprotein expression in a specific tissue or organ, can also be used to assess the role of small molecular weight selenocompounds in health. Therefore, the conditional knockout of *trsp* in specific tissues and organs is an important tool for assessing the role of selenoproteins in health and development and this model can also be used to assess the role of small molecular weight selenocompounds in protecting the animal against environmental stresses.

Transgenic/conditional knockout mouse models
Although the rescue of mice encoding a Δ*trsp* mice with G37*trsp'* provides a novel model for studying the role of housekeeping and stress-related selenoproteins in health and the role of the two Sec tRNA[Ser]Sec isoforms in governing selenoprotein expression [6,7], this mouse model permit us to focus on the animal as a whole and not on not on the roles of these components in individual organs and tissues. However, removal of selenoprotein expression by targeting floxed *trsp* with a specific promoter Cre and replacing selenoprotein expression with a mutant *trsp'* permits us to assess the role of housekeeping and stress-related selenoproteins, as well as the role of both isoforms, in health and/or proper function of specific organs or tissues. Selenoprotein removal was targeted in liver of the floxed *trsp*

mouse using the albumin *Cre* promoter and selenoprotein expression was replace with either G37*trsp^t* or A34*trsp^t* (B.A. Carlson, M.E. Moustafa, R. Shrimali, M. Rao, N. Zhong, S. Wang, L. Feigenbaum, B.J. Lee, V.N. Gladyshev and D.L. Hatfield, submitted). The pattern of replacing selenoprotein expression, wherein several selenoproteins associated with stress were not recovered, was similar with either the G37 and A34 mutant transgenes. The fact that the two mutant Sec tRNA[Ser]Sec isoforms govern selenoprotein expression in virtually an identical manner without the influence of host wild type Sec tRNA[Ser]Sec demonstrates that Um34 is responsible for the synthesis of stress-related selenoproteins.

Importantly, this mouse model will most certainly provide insights into one of the central questions in the selenium field which is "What are the contribution of selenoproteins versus low molecular weight selenocompounds in the cancer chemopreventive effects of selenium and other health benefits of this trace element?" Both these mutant transgenes replaced housekeeping selenoprotein expression, but not stress-related selenoprotein expression.

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