UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA

CARACTERIZAÇÃO DOS GENES QUE CODIFICAM SELENOPROTEÍNAS EM PEIXE-ZEBRA (*Danio rerio*)

DISSERTAÇÃO DE MESTRADO

Gabriel Teixeira de Macedo

Santa Maria, RS, Brasil

2020

CARACTERIZAÇÃO DOS GENES QUE CODIFICAM SELENOPROTEÍNAS EM PEIXE-ZEBRA (Danio rerio)

Gabriel Teixeira de Macedo

Dissertação de mestrado apresentado ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica da Universidade Federal de Santa Maria (UFSM), como requisito parcial para a obtenção do grau de **Mestre em Ciências Biológicas: Bioquímica Toxicológica.**

Orientadora: Profa. Dra. Nilda Berenice de Vargas Barbosa
Co-orientador: Prof. Dr. Daniel Mendes Pereira Ardisson-Araújo

Santa Maria, RS, Brasil

This study was financied in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) – Finance Code 001

de Macedo, Gabriel
CARACTERIZAÇÃO DOS GENES QUE CODIFICAM SELENOPROTEÍNAS EM PEIXE-ZEBRA (Danio rerio) / Gabriel de Macedo.- 2020.
72 p.; 30 cm

Orientadora: Nilda Berenice de Vargas Barbosa
Coorientador: Daniel Mendes Pereira Ardisson-Araújo
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, RS, 2020

1. Transcriptoma 2. SECIS 3. Selenocisteína 4. Selênio I. Berenice de Vargas Barbosa, Nilda II. Mendes Pereira Ardisson-Araújo, Daniel III. Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Declaro, GABRIEL DE MACEDO, para os devidos fins e sob as penas da lei, que a pesquisa constante neste trabalho de conclusão de curso (Dissertação) foi por mim elaborada e que as informações necessárias objeto de consulta em literatura e outras fontes estão devidamente referenciadas. Declaro, ainda, que este trabalho ou parte dele não foi apresentado anteriormente para obtenção de qualquer outro grau acadêmico, estando ciente de que a inveracidade da presente declaração poderá resultar na anulação da titulação pela Universidade, entre outras consequências legais.

UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA

A Comissão Examinadora, abaixo assinada,
aprova a Dissertação de Mestrado

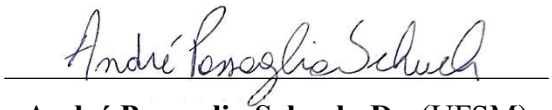
CARACTERIZAÇÃO DOS GENES QUE CODIFICAM SELENOPROTEÍNAS EM PEIXE-ZEBRA (*Danio rerio*)

elaborada por
Gabriel Teixeira de Macedo

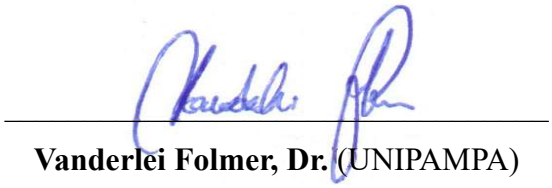
como requisito parcial para obtenção do grau de
Mestre em Ciências Biológicas: Bioquímica Toxicológica

COMISSÃO EXAMINADORA:

Nilda Berenice de Vargas Barbosa, Dra
(Presidente/Orientador)



André Passaglia Schuch, Dr. (UFSM)



Vanderlei Folmer, Dr. (UNIPAMPA)

Santa Maria, 21 de fevereiro de 2020.

DEDICATÓRIA

À minha vó Elba.

AGRADECIMENTOS

Agradeço a minha orientadora Nilda, pela ajuda em todas as horas, também ao meu coorientador Daniel, aos meus colegas de laboratório, aos colegas da Bioquímica. Agradeço à minha família e a meus amigos. Por fim, agradeço à Universidade Federal de Santa Maria pela oportunidade de ter uma educação gratuita e de qualidade.

Há uma teoria que indica que sempre que qualquer um descobrir exatamente o que, para que e porque o universo está aqui, o mesmo desaparecerá e será substituído imediatamente por algo ainda mais bizarro e inexplicável... Há uma outra teoria que indica que isto já aconteceu.

- Douglas Adams (1985)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

CARACTERIZAÇÃO DOS GENES QUE CODIFICAM SELENOPROTEÍNAS EM PEIXE-ZEBRA (*Danio rerio*)

AUTOR: Gabriel Teixeira de Macedo
ORIENTADORA: Profa. Dra. Nilda Berenice de Vargas Baborsa
CO-ORIENTADOR: Prof. Daniel Mendes Pereira Ardisson-Araújo

As selenoproteínas são proteínas contendo selenocisteína (Sec) que exibem inúmeras funções fisiológicas, incluindo manutenção da homeostase redox, metabolismo dos hormônios da tireóide, transporte de selênio e atividades do tipo chaperona. Comparado a outros vertebrados, o peixe-zebra apresenta o maior número de genes codificadores de selenoproteínas identificados até o momento, e quase todas as selenoproteínas encontradas em humanos estão presentes nos peixes. Apesar disso, a variedade de selenoproteínas no peixe-zebra é pouco explorada. Para identificar alvos em potencial para estudos translacionais, foi analizado o *status* transcricional dos genes de selenoproteínas em diferentes tecidos e estágios da vida do peixe-zebra. Usando o conjunto de dados RNA-seq dos experimentos em Sequence Reads Archive (SRA) publicados no DNA Data Bank of Japan (DDBJ), foram determinados os níveis transcricionais das 37 sequências codificadoras de selenoproteínas em cérebro, coração, brânquias, fígado e músculo esquelético, comparando-os ao longo de diferentes idades. Os genes que codificam as selenoproteínas de peixe-zebra foram confirmados pela presença da estrutura SECIS na região não traduzida 3' dos mRNAs que codificam a selenoproteínas. Dos 37 genes das selenoproteínas analisados aqui, não foi encontrada uma sequência SECIS para o gene da selenoproteína J (*selenoj*). No geral, as consultas ao transcriptoma mostraram que todos os genes de selenoproteínas são ativamente transcritos nos tecidos analisados, com exceção do gene para selenoproteína E (*selenoe)*. A pesquisa com RNA-seq revelou também que o cérebro é o tecido com maior diversidade de selenoproteínas, e que a transcrição de 20 genes varia de acordo com a idade, atingindo um pico no nível de transcrição no cérebro de peixe-zebra aos 16 meses de vida. Coletivamente, os dados do presente estudo identificam o peixe-zebra e, especialmente, o cérebro de peixes-zebra adultos como potencial tecido modelo para estudos sobre selênio e selenoproteínas, focando particularmente nos genes *dio2, gpx1a, gpx4a, gpx4b, msrb1b, selenof, selenop, selenot1a, selenot2 selenow1* e *selenow2a* como alvos em futuros estudos translacionais.

Palavras-chave: Transcriptoma, SECIS, selenocisteína, selênio.

ABSTRACT

CHARACTERIZATION OF SELENOPROTEIN CODING GENES IN ZEBRAFISH (*Danio rerio*)

AUTHOR: Gabriel Teixeira de Macedo
ADVISOR: Dra. Nilda Berenice de Vargas Barbosa
CO-ADVISOR: Prof. Daniel Mendes Pereira Ardisson-Araújo

Selenoproteins are selenocysteine (Sec)-containing proteins that exhibit numerous physiological functions, including redox homeostasis maintenance, thyroid hormones metabolism, selenium transport and chaperone-like activities. Compared to other organisms, zebrafish presents the highest number of selenoprotein-coding genes identified to date, and almost all selenoproteins found in human are present in the fish. Despite this, the variety of selenoproteins in zebrafish is little explored. In order to identify potential targets for translational studies, herein we analyzed the transcriptional status of selenoprotein genes in different tissues and life stages of zebrafish. Using RNA-seq data set from Sequence Reads Archive (SRA) experiments published at the DNA Data Bank of Japan (DDBJ), we determined the transcriptional levels of the 37 selenoproteins-coding sequences in brain, heart, gills, liver and skeletal muscle, comparing them throughout aging. Zebrafish selenoprotein-coding genes were confirmed by the presence of 3'-UTR SECIS structure in selenoprotein-coding mRNAs. Out of 37 selenoprotein genes analyzed here, we were unable to found a proper SECIS sequence for Selenoprotein J gene (*selenoj*). Overall, our transcriptome queries suggest all selenoprotein genes are actively transcribed in the tissues, with the exception of Selenoprotein E gene (*selenoe*). Finally, RNA-seq search revealed brain has the highest selenoprotein diversity and the transcription of 20 genes varied accordingly to age, with a peak at 16-month-old zebrafish brain. Collectively, the data from the present study support zebrafish and specially zebrafish adult brain as potent model organism for studies towards selenium and selenoproteins, focusing particularly on *dio2, gpx1a, gpx4a, gpx4b, msrb1b, selenof, selenop, selenot1a, selenot2 selenow1,* and *selenow2a* genes as targets for future translational studies.

Keywords: Transcriptome, SECIS, selenocystein, selenium, zebrafish.

LISTA DE FIGURAS

REVISÃO BIBLIOGRÁFICA

MANUSCRITO

SUMÁRIO

APRESENTAÇÃO

No item **INTRODUÇÃO** está descrita uma revisão bibliográfica sucinta sobre os temas abordados nesta dissertação. No final deste item estão apresentados a **justificativa** do trabalho e os **objetivos geral e específicos**.

O **DESENVOLVIMENTO** está disposto na forma de um manuscrito, o qual se encontra no item **MANUSCRITO**. As seções Introdução, Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se no manuscrito e representam a íntegra deste estudo.

No item **CONCLUSÕES** são apresentadas as conclusões gerais do presente trabalho.

As **REFERÊNCIAS BIBLIOGRÁFICAS** apresentadas no final da dissertação referem-se somente às citações que aparecem ao item INTRODUÇÃO.

1 INTRODUÇÃO

1.1 SELÊNIO

O selênio como elemento foi descoberto em 1817 por Jöns Jacob Berzelius enquanto examinava um sedimento de uma mineração de ácido sulfúrico em Gripsholm, Suécia. A escolha do nome foi em referência à deusa grega da lua, Selene; contrastando com a nomeação do elemento com características semelhantes, Telúrio, o qual referencia a deusa grega do Planeta Terra, Tellus (TROFAST, 2011). O elemento foi descrito a partir de um mineral conhecido como "telúrio sueco", e suas características elementares entre enxofre e telúrio foram documentadas (OLDFIELD, 1974). O selênio está localizado no grupo 16 da tabela periódica e faz parte da família do oxigênio, enxofre, telúrio e polônio (HOUSECROFT & SHARPE, 2012).

A toxicidade do selênio foi reportada ainda no século XIV (JAPHA, 1842); no entanto, somente após um século observada em animais de criação pecuária diagnosticados com "doença de álcali", depois caracterizada como um tipo de selenose. Diversos sintomas foram relacionados ao consumo de selênio em altas concentrações em cereais de cultivo, forrageio animal e plantas acumuladoras de selênio (ROBINSON, 1933). Perda de pêlos e danos nos cascos eram alguns dos efeitos adversos da ingestão contínua de selênio orgânico (ROSENFELD & BEATH, 1964).

Algum tempo após a definição do selênio como um elemento tóxico para os seres vivos, houve uma mudança de paradigma e os estudos se voltaram para os benefícios fisiológicos da ingestão do selênio como microelemento essencial. O caráter essencial do selênio foi descrito pela primeira vez em *Escherichia coli* (PINSENT, 1954) depois em ratos que apresentavam necrose hepática e deficiência de selênio (SCHWARZ & FOLTZ, 1957). Pesquisas quanto à deficiência de selênio levaram à ligação entre o elemento e diversas doenças em animais (TAN et al., 1979). A Doença de Keshan foi descrita em humanos como um tipo de cardiomiopatia associada com a deficiência

de selênio (TAN et al., 1979). A disponibilidade natural de selênio no solo tem uma grande variabilidade dependendo da região (DUMONT et al., 2006). Para combater a Doença de Keshan e Kashin-Beck em áreas com baixo teor de selênio no solo, na China há a adição de selenito de sódio no sal de mesa (KESHAN DISEASE RESEARCH GROUP, 1979). A inclusão de selênio em fertilizantes é obrigatória na Finlândia por suspeita de que o alto índice de doenças cardiovasculares no país esteja relacionado com os baixos níveis de selênio encontrados na população (KOIVISTOINEN & HUTTUNEN, 1986).

Estudos sobre a toxicidade do selênio evidenciaram, pela primeira vez, a presença do elemento em proteínas e em um composto similar ao aminoácido cisteína (Cys) (FRANKE & PAINTER, 1935). Flohe et al. (1973) confirmaram a importância do selênio quando o identificaram como parte essencial da glutationa peroxidase (GPx), tratando-a como uma selenoenzima por usar o selênio como um "co-fator". Pouco depois, Cone et al. (1976) publicaram a descoberta do 21° aminoácido selenocisteína (Sec). O papel fisiológico do selênio está associado aos grupamentos selenois (-SeH) no aminoácido Sec inserido em proteínas chamadas selenoproteínas. O selênio em -SeH mimetiza parcialmente o enxofre em grupamentos tióis (-SH) encontrados no aminoácido Cys (ROCHA et al., 2017).

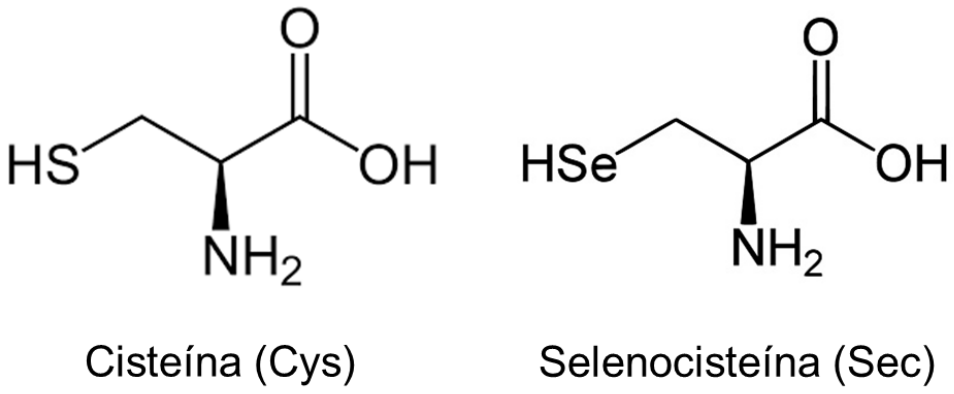


Figura 1. Estrutura química dos aminoácidos Cys e Sec.

1.2 SELENOPROTEÍNAS

A biossíntese de selenoproteínas é essencial para a vida (BÖSL et al., 1996), e requer primeiramente a conversão do selênio da dieta para seleneto (Se^{2-}). Formas inorgânicas como selenito (SeO_3^{2-}) e selenato (SeO_4^{2-}) são convertidas através das vias glutationa-glutarredoxina e tiorredoxina. Formas orgânicas como selenometionina e selenocisteína são metabolizadas a Se2- pela selenocisteína liase ou por trans-selenação. Somente Se^{2-} pode, então, ser fosfatado pela enzima selenofosfato sintetase-2 (SEPHS2), e formar selenofosfato ($SePO_3^{3-}$) que irá doar selênio para Sec. O RNA transportador (tRNA) que irá transportar Sec está, originalmente, ligado a uma serina (Ser), que é fosforilada pela enzima fosfosseril-tRNA quinase, convertida a Sec e desfosforilada pela enzima O-fosfosseril-tRNA[Sec] selênio transferase. O produto é o selenocisteinacil-tRNA de selenocisteína (Sec-tRNA[Ser]Sec) que é posteriormente recrutado durante a tradução. O processo de síntese de selenoproteínas, como descrito por Labunskyy et al. (2014), está representado na Figura 2, adaptada de Cardoso et al. (2015).

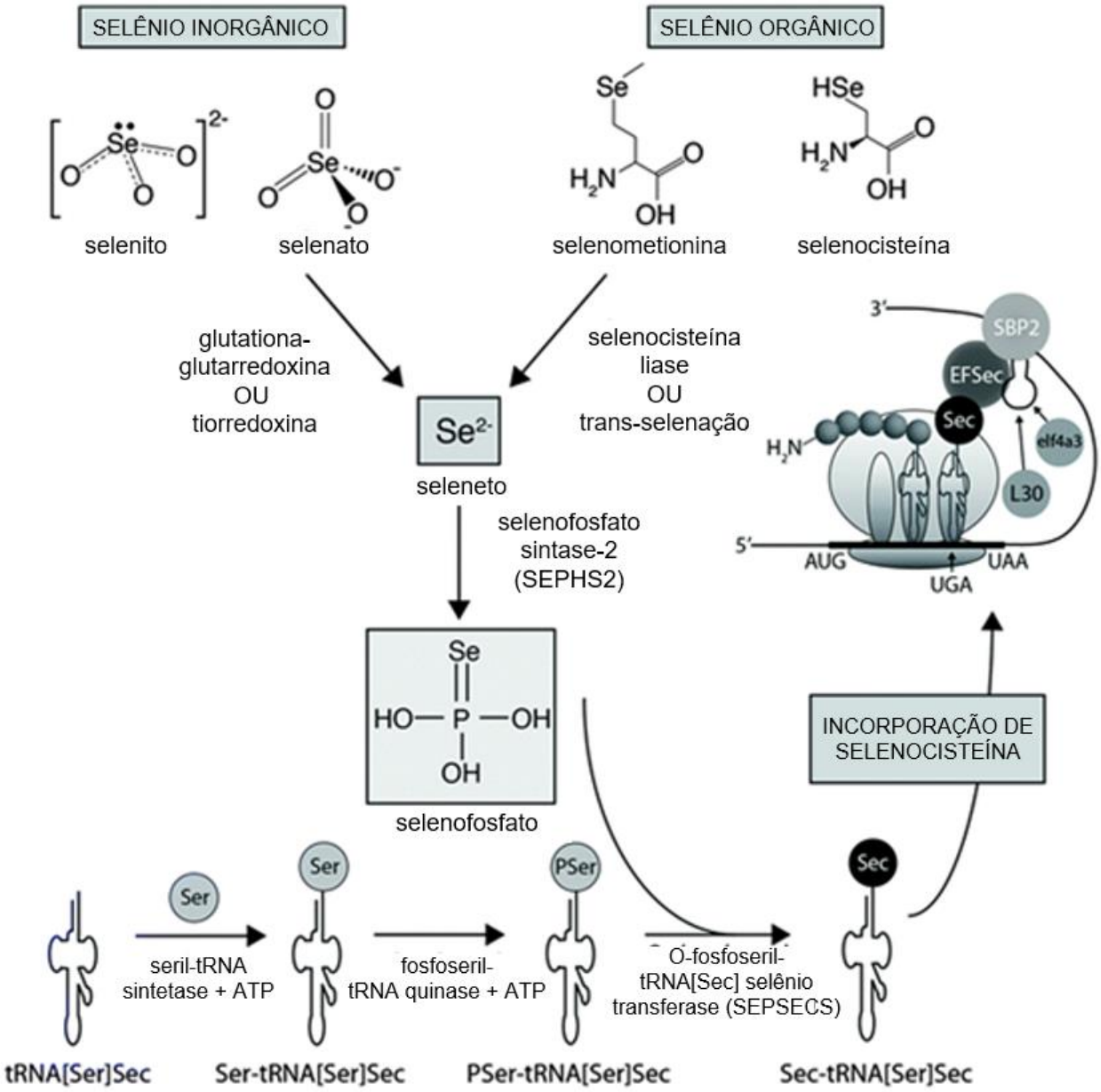


Figura 2. Via da síntese de selenoproteínas. Fontes orgânicas e inorgânicas de selênio transformadas em seleneto, que é fosfatado pela SEPHS2 e doa Se ao tRNA. Sec-tRNA[Ser]Sec é recrutado pelas proteínas que interagem com o elemento SECIS do mRNA modificado para a incorporação de Sec na tradução. Adaptado de Cardoso et al. (2015).

Para a tradução de selenoproteínas ocorrer, o resíduo Sec deve ser codificado através do códon UGA, um dos três códons que indicam terminação da tradução em situação normal. Para que a terminação não aconteça onde Sec deve ser introduzido, o RNA mensageiro (mRNA) transcrito por genes de selenoproteínas deve conter um elemento com sequência de inserção de Sec

(SECIS) na região não traduzida 3' (3' UTR) que funciona como uma plataforma para proteínas que se ligam ao RNA (HATFIELD & GLADYSHEV, 2002).

Os elementos SECIS são sequências de nucleotídeos que adquirem uma estrutura secundária chamada stem-loop, por conta da ligação entre as bases nitrogenadas na região stem (haste) e a não-ligação de bases na região loop (laço). A sequência de SECIS em eucariotos mantém duas regiões conservadas: o núcleo SECIS, composto pelo quarteto não-Watson-Crick após o primeiro loop, e uma dupla ou trio de adenina no segundo loop. As diferenças entre as sequências núcleotídicas nas regiões stem e/ou loop podem modular a eficiência de inserção de Sec e um elemento SECIS pode ser milhares de vezes mais eficaz que outro (LATRÉCHE, et al., 2009). Elementos SECIS podem ser divididos em dois tipos como demonstrado na Figura 3, adaptado de Latréche et al., 2009. O elemento SECIS irá se complexar com o fator de elongação específico para Sec (EFSec) e com a proteína de ligação a SECIS 2 (SBP2). Logo, EFSec interage com SBP2 para recrutar o Sec-tRNA[Ser]Sec e mediar a inserção do aminoácido no peptídeo em formação (HATFIELD & GLADYSHEV, 2002). Outras proteínas que interagem com SECIS foram encontradas como a proteína ribossomal L30 (BIFANO et al., 2013) e um fator de iniciação eucariótico (elF4a3) que está relacionado com o status de selênio e com a expressão diferencial de selenoproteínas (BUDIMAN, 2009). A nucleolina também se liga ao SECIS e provavelmente participa da ligação com o ribossomo (WU et al., 2000). O processo de incorporação da selenocisteína está representado na Figura 2, adaptado de Cardoso et al. (2015).

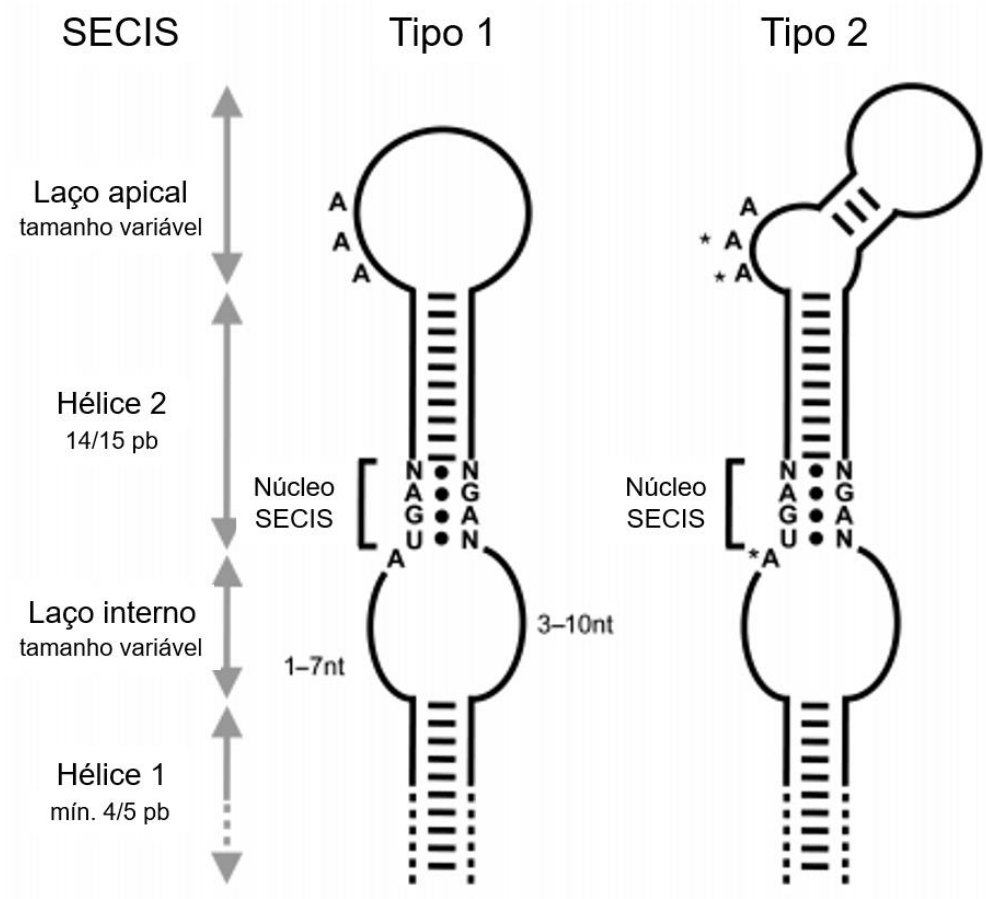


Figura 3. Estrutura secundária formada pela sequência nucleotídica em elementos SECIS. Divisão em laços e hélices com destaque para estruturas conservadas: o núcleo SECIS e o tamanho dos elementos internos.

O status de selênio no organismo altera a expressão de selenoproteínas, e existe uma hierarquização entre as selenoproteínas que são mais ou menos afetadas, assim como os tecidos. Sob restrição de selênio, a expressão de genes não é igualmente afetada para todas as selenoproteínas (SUNDE et al., 2009). A célula possui mecanismos complexos para destruir mRNA de selenoproteínas através da degradação de mRNA mediada por mutação sem sentido (NMD, do inglês nonsense-mediated decay) evitando a tradução com um códon de terminação prematuro (KERVESTIN & JACOBSON, 2012). Esses mecanismos estão relacionados com a afinidade entre a SBP2 e o elemento SECIS (LOW et al., 2000). Portanto, a posição do gene na

hierarquia de selenoproteínas pode estar codificada na sequência do seu elemento SECIS. A regulação de selenoproteínas também depende do tecido. Em situação de escassez de selênio, o fígado e outros tecidos se esgotam do elemento antes do cérebro, indicando que pressões evolutivas modularam a preferência de certos órgãos (BURK & HILL, 2015).

Até o final do século XX, a descoberta de novas selenoproteínas era realizada por experimentos de bancada, e 12 selenoproteínas de mamíferos haviam sido caracterizadas (HOLBEN & SMITH, 1999). A descrição de novas selenoproteínas em diferentes organismos aumentou com o avanço das ferramentas computacionais e da quantidade de dados acumulados no começo do século XXI. Lescure et al. (1999) descreveram três novas selenoproteínas utilizando metodologias de predição de estruturas secundárias de mRNA, ao mesmo tempo que Kryukov et al. (1999) desenvolviam SECISearch, um software para procura de elementos SECIS termodinamicamente estáveis em mRNA. Pela primeira vez, um genoma completo foi analisado em busca de elementos SECIS e códons de terminação foram anotados corretamente por Martin-Romero et al. (2001) e Castellano et al. (2001) no genoma de *Drosophila melanogaster*. O método foi aplicado a medida que os genomas humanos e de roedores foram publicados, e foi constatado uma variação grande de selenoproteínas entre as espécies. Diferentes espécies de Drosophila têm entre 0 e 3 selenoproteínas (CHAPPLE & GUIGO, 2008). Selenoproteínas estão presentes nos três domínios da vida, mas há grupos inteiros de organismos sem selenocisteína como plantas terrestres, Lepidoptera e a maior parte dos fungos (LOBANOV et al., 2008). A alga unicelular *Aureococcus anophagefferens* é o organismo com mais selenoproteínas, com 59 proteínas diferentes contendo Sec (GLOBER et al., 2013).

As selenoproteínas em vertebrados apresentam diversas funções e estão distribuídas em diferentes famílias. Algumas das selenoproteínas com funções melhor elucidadas são: glutationas

peroxidases (GPX) e tiorredoxina redutase (TXNRD), com função antioxidante; iodotironina desiodinases (DIO), reguladoras da síntese de hormônios tireoidianos; selenoproteína P (SELENOP), transportadora de selênio e SEPHS2, que participa da síntese de Sec (PAPP et al., 2007). Entre as proteínas menos conhecidas, algumas são antioxidantes, sinalizadoras de oxidorredução (ou redox), ou ainda com função tipo-chaperona (PAPP et al., 2007). A biodisponibilidade de selênio varia entre o ambiente terrestre e aquático, pois o selênio do solo acaba acumulando em corpos d'água e bioacumulando através da cadeia trófica aquática (SELINUS et al., 2005). A medida que os vertebrados evoluíram para fora da água, a deficiência de selênio se tornou um potencial fator seletivo, e a persistente conservação de Sec mostra que o resíduo não é facilmente substituído por Cys, apesar de acontecer em mais de uma ocasião (CASTELLANO et al., 2009).

1.3 PEIXE-ZEBRA

Entre os teleósteos, o peixe-zebra (*Danio rerio*) é um organismo modelo amplamente utilizado em pesquisas das mais diversas áreas. O peixe-zebra tem o genoma totalmente mapeado, apresenta grande sensibilidade a fármacos, um rápido metabolismo, e genes evolutivamente conservados com alto grau de similaridade comparado a genes humanos e de camundongos (BARBAZUK et al., 2000; GOLDSMITH, 2004; HOWE et al., 2013). Fácil manipulação, baixo custo, comportamento facilmente observado em um ambiente controlado e rápido desenvolvimento e ciclo biológico também são vantagens importantes do uso do animal em experimentação (SHIN & FISHMAN, 2002; LIESCHKE & CURRIE, 2007).

Estudos realizados com selenoproteínas de peixe-zebra reportam uma variabilidade dependente de cepa e sexo (BENNER et al., 2010; DREW et al., 2012). Também há evidências que há resposta na expressão de selenoproteínas dependente do status de selênio e balanço redox (BENNER et al., 2013; PENGLASE et al., 2014a; PENGLASE et al., 2014b; BETANCOR et al.,

2015; DOLGOVA et al., 2019). Em peixes ósseos, houve uma abundância de duplicações gênicas junto a um aumento da dependência ao selênio ambiental, indo na contra-mão do observado em vertebrados terrestres (REICH & HONDAL, 2016). Apesar disso, a variedade de selenoproteínas que o peixe-zebra apresenta é pouco explorada, não sendo foco de estudos a diferenciação de função em duplicações de genes com homólogos em mamíferos e/ou genes exclusivos de peixes. O aprofundamento de estudos sobre os genes que codificam selenoproteínas de peixe-zebra pode fortalecer o uso do peixe-zebra como modelo interessante para o estudo do metabolismo de selênio em vertebrados.

2 JUSTIFICATIVA

Embora o selênio seja um elemento traço essencial, é muito estreito o limiar entre a janela considerada terapêutica e tóxica; e seus efeitos ora tóxicos, ora benéficos ainda fazem com que seja entendido como um "veneno essencial" (JUKES, 1983). Neste cenário, o estudo das selenoproteínas representa um campo altamente importante para o delineamento de pesquisas com enfoque no elemento tanto em termos farmacológicos como toxicológicos. Como mencionado anteriormente, ao contrário dos vertebrados terrestres, os teleósteos tornaram-se mais dependentes de selênio, característica que pode servir como plataforma para o estudo do elemento e das selenoproteínas. Este estudo de diferentes selenoproteínas irá mapear, pela primeira vez juntos, os genes, transcritos e proteínas de peixe-zebra. O conjunto de dados poderá fornecer informações específicas que podem ser usadas para delinear estudos experimentais com foco em selênio em nível de entidadade biológica e/ou translacional.

3 OBJETIVOS

3.1 Objetivo geral

Caracterizar em nível molecular selenoproteínas em peixe-zebra a fim de identificar na espécie potenciais genes, tecidos e idades alvos para estudos translacionais.

3.2 Objetivos específicos

- Analisar a distribuição cromossômica de genes que codificam para selenoproteínas no genoma de peixe-zebra;
- Identificar e comparar os elementos SECIS de cada gene de selenoproteínas em peixe-zebra;
- Analisar por RNA-seq a transcrição de genes de selenoproteínas em diferentes tecidos de peixes adultos, incluindo cérebro, coração, brânquias, fígado e músculo esquelético em diferentes fases do peixe, aos 2, 7, 16 e 39 meses de vida;

4 MANUSCRITO

O manuscrito resultante desta dissertação será submetido a um periódico internacional e encontra-se abaixo em sua integralidade.

TRANSCRIPTIONAL PROFILE OF SELENOPROTEIN-CODING GENES IN FOUR DIFFERENT ZEBRAFISH TISSUES THROUGHOUT AGING

Gabriel T. de Macedo[1], Matheus M. dos Santos[1], Bruna C. Piccoli[1], João B. T. Da Rocha[1], Daniel M. P. Ardisson-Araújo[1], Nilda B. V. Barbosa[1]*

[1] Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Santa Maria, RS, Brasil.

* Corresponding author: Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil. nvbarbosa@yahoo.com.br.

TRANSCRIPTIONAL PROFILE OF SELENOPROTEIN-CODING GENES IN FOUR DIFFERENT ZEBRAFISH TISSUES THROUGHOUT AGING

ABSTRACT

Selenoproteins are selenocysteine (Sec)-containing proteins that exhibit numerous physiological functions, including redox homeostasis maintenance, thyroid hormones metabolism, selenium transport and chaperone-like activities. Compared to other organisms, zebrafish presents the highest number of selenoprotein-coding genes identified to date, and almost all selenoproteins found in human are present in the fish. Despite this, the variety of selenoproteins in zebrafish is little explored. In order to identify potential targets for translational studies, herein we analyzed the transcriptional status of selenoprotein genes in different tissues and life stages of zebrafish. Using RNA-seq data set from Sequence Reads Archive (SRA) experiments published at the DNA Data Bank of Japan (DDBJ), we determined the transcriptional levels of the 37 selenoproteins-coding sequences in brain, heart, gills, liver and skeletal muscle, comparing them throughout aging. Zebrafish selenoprotein-coding genes were confirmed by the presence of 3'-UTR SECIS structure in selenoprotein-coding mRNAs. This work presents, therefore, the most comprehensive selenoprotein genes and transcript characterization up to now. Out of 37 selenoprotein genes analyzed here, we were unable to found a proper SECIS sequence for Selenoprotein J gene (*selenoj*). Overall, our transcriptome queries suggest all selenoprotein genes are actively transcribed in the tissues, with the exception of *selenoe* gene. Finally, RNA-seq search revealed brain has the highest selenoprotein diversity and the transcription of 20 genes varied accordingly to age, with a peak at 16-month-old zebrafish brain. Collectively, the data from the present study support zebrafish and especially adult brain as potent models for studies towards selenium and selenoproteins, specifically targeting *dio2, gpx1a, gpx4a, gpx4b, msrb1b, selenof, selenop, selenot1a, selenot2 selenow1,* and *selenow2a* genes in future translational studies.

Keywords: Transcriptome, SECIS, selenocystein, selenium, zebrafish.

1 INTRODUCTION

The element selenium (Se) is located in the group 16 from periodic table, along with oxygen, sulfur, tellurium and polonium (HOUSECROFT & SHARPE, 2012). The essential characteristic of Se for life was firstly described in *Escherichia coli* (PINSENT, 1954) and, later in rats that developed liver necrosis due to Se deficiency (SCHWARZ & FOLTZ, 1957). Nowadays, Se is recognized as a trace element for animals due to its inclusion in the 21st amino acid selenocysteine (Sec), which is incorporated in proteins called selenoproteins.

The biosynthesis of selenoproteins primarily requires the conversion of dietary selenium to selenide (Se^{2-}), which is phosphated by the enzyme selenophosphate synthase-2 (Sephs2) to form selenophosphate ($SePO_3^{3-}$) that will donate selenium to Sec. For selenoprotein translation, the Sec residue is encoded using the UGA codon, one of the three codons that indicate termination of the translation in a normal situation. For the termination not happens where Sec is introduced, the messenger RNA (mRNA) transcribed by selenoprotein genes contains an element with Sec insertion sequence (SECIS) in the 3 '(3' UTR) untranslated region that functions as a platform for proteins that bind to RNA (HATFIELD & GLADYSHEV, 2002).

It is known that Se status can alter the selenoprotein expression pattern, and that there is a hierarchy among the selenoproteins that are more or less affected, as well as the tissues (SUNDE et al., 2009). In a situation of scarcity of selenium, brain has higher priority for Se than the peripheral tissues, indicating that evolutionary pressures modulated the preference for certain organs (BURK & HILL, 2015).

Until the end of the 20th century, the discovery of new selenoproteins was carried out by bench experiments, and 12 mammalian selenoproteins had been characterized (HOLBEN & SMITH, 1999). The description of new selenoproteins in different organisms has increased with the advancement of computational tools and the amount of data accumulated in the beginning of the 21st century. Lescure et al. (1999) described three new human selenoproteins using methodologies for predicting secondary mRNA structures and, in the same year the SECISearch, a software for searching for thermodynamically stable SECIS elements in mRNA was developed by Kryukov et al. (1999).

Currently, it is known that selenoproteins are present in the three domains of life, but there are entire groups of organisms without selenocysteine such as terrestrial plants, lepidoptera and most fungi (LOBANOV et al., 2008).

25 different selenoproteins with varied functions are present in humans and are classified in different families. The selenoproteins with well elucidated biological role include the glutathione peroxidases (GPx), and thioredoxin reductases (TxnRd) with antioxidant functions; the iodothyronine deiodinases (Dio) regulating the synthesis of thyroid hormones, the selenoprotein P (SELENOP) as selenium transporter, and the Sephs2, that participates in the synthesis of Sec (PAPP et al., 2007). Among those with less recognized functions there are redox regulators and chaperone-like proteins (PAPP et al., 2007).

Se bioavailability varies between the terrestrial and aquatic environment, since the element in the soil ends up accumulating in water bodies and bioaccumulating through the aquatic food chain (SELINUS et al., 2005). As vertebrates evolved out of the water, selenium deficiency became a potential selective factor, and the persistent conservation of Sec shows that the residue is not easily replaced by Cys, despite occurring on more than one occasion (CASTELLANO et al., 2009). Among teleosts, zebrafish (*Danio rerio*) is a model organism widely used in the most diverse research areas. Zebrafish has a fully mapped genome, a great sensitivity to drugs, a fast metabolism, and evolutionarily conserved genes with a high degree of similarity with human and mouse genes (BARBAZUK et al., 2000; GOLDSMITH, 2004; HOWE et al., 2013).

Studies conducted with zebrafish selenoproteins report that the variability is strain and sex dependent (BENNER et al., 2010; DREW et al., 2012). There is also evidence that the selenoprotein expression depends on Se status and redox balance, but we have no knowledge of aging influence on selenoprotein transcription (BENNER et al., 2013; PENGLASE et al., 2014a; PENGLASE et al., 2014b; BETANCOR et al., 2015; DOLGOVA et al., 2019).

Differently of terrestrial vertebrates, in bone fish there was an abundance of gene duplications with an increased dependence on environmental selenium (REICH & HONDAL, 2016). Despite this, the variety of selenoproteins present in zebrafish is little explored, and the differentiation of function in gene duplication with homologues in mammals and/or exclusive fish genes is not usually focus of studies. So, researches on genes that encode zebrafish selenoproteins may strengthen the use of zebrafish as an interesting model for studying Se homeostasis in vertebrates. With this in mind, the aim of this study was to characterize selenoproteins in zebrafish at the

transcriptional level in order to identify potential targets for translational studies. Firstly, we identified the zebrafish genes that code for selenoproteins and the presence or absence of homologues in other organisms. Then, we analyzed the transcriptional status of selenoprotein genes in different tissues of adult zebrafish by RNA-seq, including brain, heart, gills, liver and skeletal muscle. To ascertain the impact of aging on selenoprotein-coding gene transcription levels, we also analyzed selenoprotein transcripts in 2-, 7-, 16- and 32-months old zebrafish. Our results indicating that the transcriptional status of selenoprotein genes is higher and more diversified in zebrafish brain, along with an overall higher transcription in adult phase, make adult brain a potential tissue to investigate the effects of dietary selenium on selenoproteins modulation. Considering identity to human sequences, transcription level and known function, we point *dio2, gpx1a, gpx4a, gpx4b, msrb1b, selenof, selenop, selenot1a, selenot2 selenow1,* and *selenow2a* as potential targets for future selenoprotein studies in zebrafish.

2 MATERIALS AND METHODS

2.1 RNA-seq acquisition

Transcriptome data of dissected zebrafish tissues at different development stages were generated by Kijima et al. (2018). Each group consisted of five fish and the experiments were performed by Illumina HiSeq 2000 paired end sequencing method. The RNA-seq data were obtained from the Japanese DNA data bank (DDBJ) in fastq format (Access Number: PRJDB7713). The files were imported into Geneious 9.0 software and trimmed to remove low-quality regions with more than 5% base error chance. The deep-sequencing analyses included brain, gill, cardiac muscle (heart), liver, and skeletal muscle (muscle) of five different zebrafish individuals at 2, 7, 16 and 39-months-old. Importantly, zebrafish sex distribution and strain were not specified and the heart samples were not collected from 2-months-old fishes due to dissection difficulties. In total, each paired-end sequence generated two different technical replicates. For brain, the influence of the age on selenoprotein transcription were analyzed in larval, adult, and senescent fishes. Zebrafish selenoproteins and their functions are listed in the Table 1. This paper follows nomenclature as agreed by HUGO Gene Nomenclature Committee (GLADYSHEV et al., 2016), which settle *Seleno* as common prefix instead of *Sel* or *Sep*. In addition, zebrafish default nomenclature was employed to proteins with uppercase and to genes with lowercase italic.

2.2 Transcriptomic analysis

Coding DNA sequence (CDS) for 37 zebrafish selenoprotein transcript sequences (Table 1) were utilized as target to quantify the selenoprotein transcription. In genes with multiple variants, the varying exons were analyzed separately. Two housekeeping gene sequences were used as transcription control for each group: *rpl13a* (Ribosomal Protein L13a) and *uba52* (Ubiquitin A-52 Residue Ribosomal Protein Fusion Product 1). These genes have been utilized as reference in selenoprotein transcription studies and recommended as reference genes in multiple species (Sadritdinova et al, 2014; Bian et al, 2015). Geneious 9.0 'Map to Reference' function was used to map each trimmed RNA-seq to a reference sequence. Mapping sensitivity was set as 99% minimum overlap identity and 20% maximum mismatches per read. Fine tuning iterated the process three times and utilized the repeat with more matches. Abundance of transcripts was quantified as the number of aligned nucleotides between each selenoprotein gene CDS and RNA-seq sequences. The normalization by control data was carried out by dividing the total matched nucleotide by the total nucleotides in the experiment sequences and the target sequence size to avoid size bias.

2.4 Prediction of SECIS sequences and chromosome loci identification

We analyzed the SECIS element for each zebrafish gene with at least one Sec residue. For each selenoprotein encoding mRNA sequence, the SECISearch3 search method (Mariotti et al., 2013) was applied using SECISearch3.0 / Seblastian online tool for prediction of eukaryotic SECIS elements (available at: http://seblastian.crg.es). The predicted SECIS was compared with previously noted regions in the reference genome assembly. The sequence of SECIS elements for all genes was aligned with Multiple Alignment using Fast Fourier Transform (MAFFT) (Katoh et al., 2002) and conserved regions were described. The graphic visualization of genes localization in the zebrafish genome was produced using the mapping tool available in the genomic browser Ensembl 92, considering nucleotide proportions.

2.5 Statistical analysis

Transcription results were expressed in fold-change from both reference sequences averages. Analysis were performed in the GraphPad Prism 7 software. Results were expressed as average ± mean standard error and analyzed through ANOVA followed by Tukey multiple comparison test. Significant difference was considered when $p \leq 0.05$.

Table 1. Selenoprotein family classification, function, genes and reference numbers

Putative Function	Protein Family	Human Gene	ZF Gene	ZF Transcript Reference #
Hormonal (Guo et al., 2014)	Iodothyronine deiodinase	*dio1*	*dio1*	NM_001007283
		dio2	*dio2*	NM_212789
		dio3	*dio3a*	NM_001256003
			dio3b	NM_001177935
Antioxidant **(**Dikiy et al., 2007; Mariotti et al., 2012; Mendieta-Serrano et al., 2015)	Glutathione Peroxidase	*gpx1*	*gpx1a*	NM_001007281
			gpx1b	NM_001004634
		gpx2	*gpx2*	NM_001329759
		gpx3	*gpx3*	NM_001137555
		gpx4	*gpx4a*	NM_001007282
			gpx4b	NM_001030070
		gpx6	-	
	Methionine Sulfoxide Reductase B1	*msrb1*	*msrb1a*	NM_178288
			msrb1b	NM_001105128
	Thioredoxin reductase	*txnrd1*	*txnrd2.2*	NM_007116
		txnrd2	-	
		txnrd3	*txnrd3*	NM_007117
	Thioredoxin-like (Rdx) family	*selenoh*	*selenoh*	NM_178133
		selenot	*selenot1a*	NM_178290
			selenot1b	NM_178292
			selenot2	NM_001098487
		selenov		
		selenow	*selenow1*	NM_178287
			selenow2a	NM_194417

			selenow2b	NM_174418
	Selenoprotein N	*selenon*	*selenon*	NM_001004294
	Selenoprotein O	*selenoo*	*selenoo1*	NM_001044871
			selenoo2	NM_001348085
	Selenoprotein U	-	*selenou1a*	NM_001354553
			selenou1b	NM_001076713
Chaperone-like (Ferguson et al., 2006; Shchedrina et al., 2007; Mariotti et al., 2012)	SEP15/ selenoprotein M family	- *selenof* *selenom*	*selenoe* *selenof* *selenom*	NM_001195784 NM_178294 NM_178286
	Selenoprotein I	*selenoi*	*selenoi*	NM_001329437
	Selenoprotein K	*selenok*	*selenok*	NM_001004681
	Selenoprotein L	-	*selenol*	NM_001190382
	Selenoprotein S	*selenos*	*selenos*	NM_001045334
Structural (Castellano et al., 2005)	Selenoprotein J	-	*selenoj*	NM_001193469
Transport/ storage (O'Leary et al., 2016)	Selenoprotein P	*selenop*	*selenop*	NM_178297
			selenop2	NM_001353911
Sec biosynthesis (Kryukov et al., 2000)	Selenophosphate synthetase	*sephs2*	*sephs2*	NM_001004295
Reference genes	-	*rpl13a*	*rpl13a*	NM_212784
	-	*uba52*	*uba52*	NM_001037113

3 RESULTS

3.1 Characterization of selenoproteins in zebrafish

Danio rerio presents 37 selenoproteins distributed along 25 chromosomes (Figure 1B). The proteins play different functions described so far (Table 1), including hormonal metabolism, antioxidant, chaperone-like, and selenium-related protein biosynthesis. Almost all selenoproteins found in human are present in zebrafish, with the unique exception in SELENOV. SELENOV likely originated in Placentalia and is expressed in mammal testis (KRYUKOV et al., 2003). The abundance of selenoproteins in fish is related to the chromosome duplication, event that took place

in teleost (Glasauer & Neuhaus, 2014). Out of 37 distinct selenoprotein-coding genes in zebrafish, SELENOE, SELENOL, SELENOJ and SELENOU lack homologs in human. All selenoprotein genes found in literature were mapped in a zebrafish chromosome map (Figure 1A). We found that most of duplicated genes are in distant loci among the 25 chromosomes, excepting SELENOW2a and SELENOW2b that have a close location in the chromosome 3.

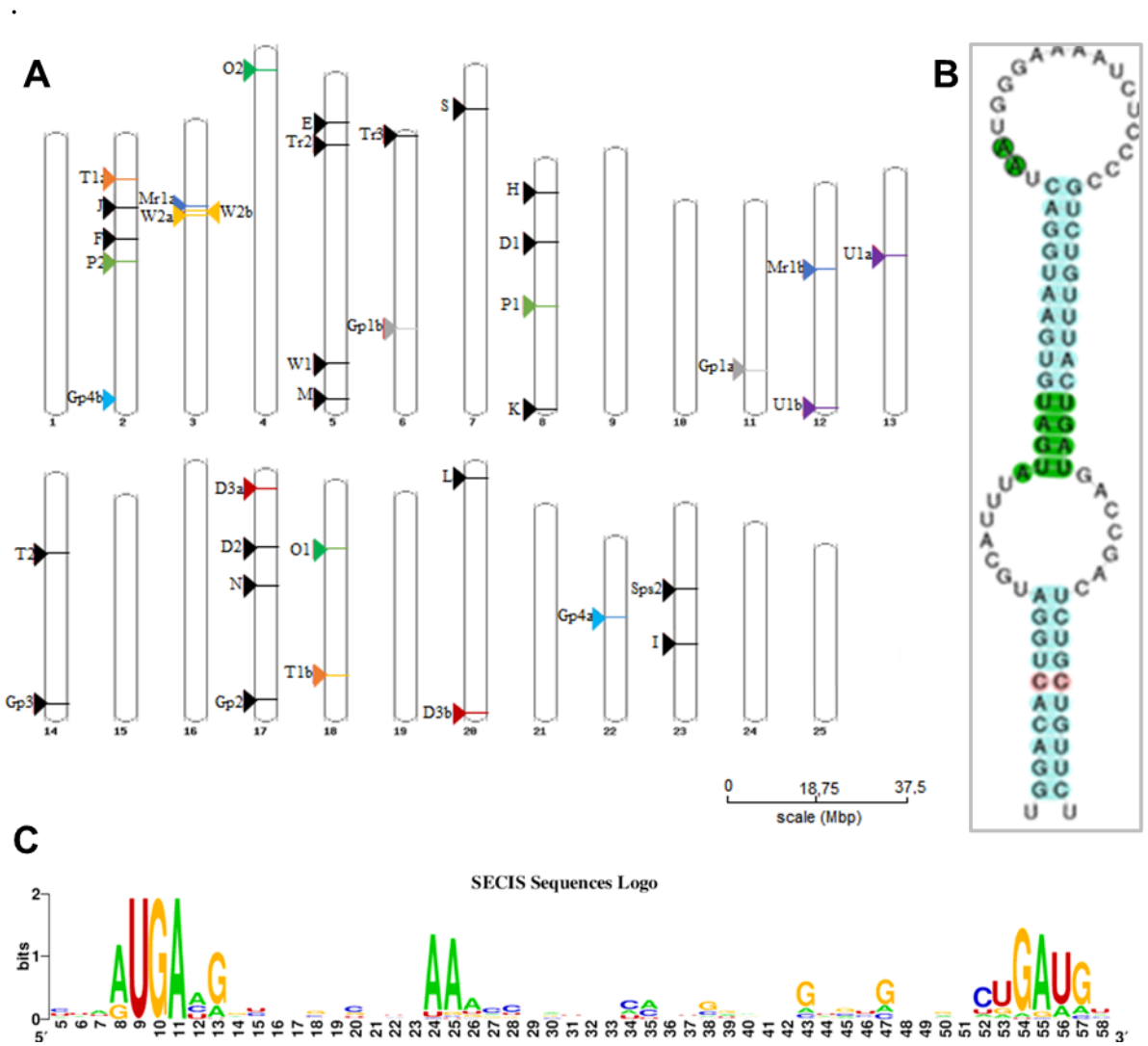


Figure 1. Selenoproteins in zebrafish. **(A)** Location of selenoprotein-coding genes in the 25 zebrafish chromosomes. Black arrows indicate gene with no recent duplication. Pairs of colored arrows indicate duplicated genes with similar nomenclature. Non-conventional abbreviation was used for better visualization (Gp = *gpx*, Tr = *txnrd*, Mr = *msrb*, Sps = *sephs*, genes with capital letter should have *seleno* prefix). Scale indicates size in million base pair. **(B)** A

secondary structure predicted for *selenoh* SECIS element (representative). **(C)** SECIS sequence logo produced with all SECIS sequences found using SECISearch 3.0 for zebrafish selenoprotein-coding gene. Large nucleotide letters indicate high nucleotide concensus.

The zebrafish selenoprotein-coding genes were confirmed by the presence of 3'-UTR Secis structure in selenoprotein-coding mRNAs. Eukaryotic SECIS elements share conserved sequences such as SECIS Core and a tridimensional structure with a characteristic loop and stem regions (Figure 1B). These characteristics in the mRNA sequences were searched using SECISearch3.0 and the results are in Supplemental Figure 1. The set of all sequences found was illustrated using WebLogo 2.8.2 (Crooks et al., 2004) (Figure 1C). We found sequences classified as type 1 SECIS element for 36 of 37 genes analyzed. No SECIS element was found in the mRNA sequence of *selenoj*. An extra SECIS sequence was found in the mRNA sequence of *dio1*. All selenoprotein transcripts size, from Start codon to SECIS location, were mapped and are represented in Supplemental Figure 2.

3.2 Transcriptome assessment in zebrafish tissues

We analyzed the selenoprotein transcriptional panorama in five different tissues of 2-, 7-, 16-, and 39-months-old zebrafish, which included brain, gill, heart, liver, and skeletal muscle. The genes *rpl13a* and *uba52* were used as controls to normalize the selenoprotein gene transcription for each tissue.

In order to identify a target age, we analyzed the number of transcripts that mapped against the 37 selenoprotein-coding transcripts along the different stages of development in zebrafish brain (Figure 2). 2- and 39-months old fishes did not present statistical difference, and the abundance of reads that mapped against the selenoprotein-coding transcripts were similar.

The abundance of reads that mapped against 7-month old fishes was higher than 2-month group and 16-months old fishes had statistically higher transcription than the other ages; therefore, we selected 7- and 16 months old for further analysis.

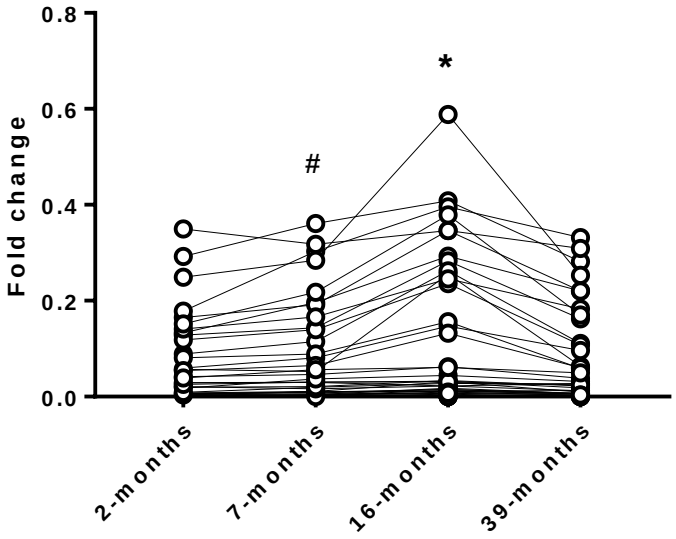


Figure 2. Zebrafish brain selenoprotein gene transcription levels at different ages in each tissue. Each point represents an average of 10 replicates for gene. *indicates significant difference from the other groups, #indicates significant difference from 2- and 16-month groups (Two-way ANOVA, $p \leq 0.05$).

Considering only 7- and 16-month old fishes, the 37 selenoprotein-coding gene transcription levels were classified as very low (0.05-fold or lower than the control), low (between 0.05 and 0.2-fold from the control) and high level (higher than 0.2-fold from control), and are presented as heat map (Figure 3). The relative selenoprotein transcription among the tissues in 7 and 16-months old zebrafish is showed in Figure 4 with the determined levels.

.

	BRAIN		GILL		HEART		LIVER		MUSCLE	
	7M	16M	7M	16M	7M	16M	7M	16M	7M	16M
dio1	0.017	0.015	0.007	0.005	0.009	0.016	0.052	0.136	8E-04	0.002
dio2	0.028	0.266	0.003	0.002	4E-05	0	0.047	0.293	2E-04	1E-03
dio3a	0	3E-04	0.001	0	4E-06	0	0	0	2E-04	0.001
dio3b	0.002	0.011	5E-04	0	1E-04	0	7E-06	0	0	0
gpx1a	0.202	0.301	0.056	0.108	0.073	0.157	0.462	0.691	0.031	0.062
gpx1b	0.01	0.007	0.032	0.003	6E-04	8E-04	0.014	0.012	8E-04	0.001
gpx2	0	4E-04	0	0.001	0	0	0	0	0	0
gpx3	0.008	0.005	1E-04	3E-04	2E-04	0	0.013	0.014	0	1E-04
gpx4a	0.338	0.291	0.026	0.023	0.018	0.055	0.953	1.809	0.007	0.012
gpx4b	0.366	0.419	0.057	0.06	0.041	0.105	0.024	0.062	0.039	0.074
msrb1a	0.005	0.01	0.002	0.004	0.004	0.013	0.011	0.017	0.004	0.007
msrb1b	0.117	0.271	0.008	0.011	0.002	0.012	0.007	0.011	0.001	0.001
selenoe	0	0	3E-05	5E-05	0	0	0.002	4E-04	2E-04	0
selenof	0.142	0.243	0.033	0.04	0.023	0.058	0.035	0.036	0.011	0.014
selenoh	0.034	0.033	0.01	0.012	0.007	0.009	0.005	0.009	0.025	0.009
selenoi	0.017	0.023	0.001	0.002	4E-04	0.002	0.002	0.003	5E-04	3E-04
selenoj	0.083	0.151	0.012	0.024	0.014	0.046	0.014	0.018	0.007	0.013
selenok	0.031	0.023	0.017	0.007	0.015	0.005	0.008	0.006	0.02	0.026
selenol	0.019	0.034	0.002	0.003	0.007	0.007	0.004	0.004	0.001	0.005
selenom	0.047	0.064	0.008	0.007	0.002	0.003	5E-04	5E-04	0.003	0.002
selenon	0.037	0.046	0.002	0.003	0.002	0.003	0.001	4E-04	0.001	0.001
selenoo1	0.011	0.011	0.004	0.002	9E-04	0.002	0.003	0.005	0.002	0.003
selenoo2	0.002	0.002	3E-05	4E-05	3E-06	0	9E-05	4E-05	5E-05	2E-05
selenop	0.368	0.406	0.085	0.052	0.107	0.109	0.196	0.343	0.078	0.197
selenop2	0	0	0	6E-05	0.004	0	0.288	0.111	0	0
selenos	0.028	0.014	0.003	0.003	0.003	0.002	0.007	0.006	0.002	0.002
selenot1a	0.168	0.253	0.022	0.028	0.008	0.033	0.025	0.045	0.014	0.022
selenot1b	0.09	0.161	0.01	0.011	0.005	0.023	0.03	0.042	0.004	0.004
selenot2	0.288	0.464	0.008	0.046	0.003	0.015	0.009	0.013	0.013	0.028
selenou1a	0.22	0.136	0.006	0.004	0.011	0.011	0.057	0.028	0.013	0.008
selenou1b	0.004	0.021	0.001	0.002	7E-05	0.002	5E-05	3E-04	0.004	0.004
selenow1	0.321	0.355	0.327	0.607	0.24	0.531	0.071	0.093	0.101	0.156
selenow2a	0.22	0.391	0.009	0.017	0.011	0.042	0.015	0.017	0.012	0.018
selenow2b	0.002	0	0.076	0.123	0	3E-04	4E-05	3E-04	0	4E-04
sephs2	0.043	0.032	0.004	0.005	0.003	0.004	0.011	0.012	0.004	0.005
txnrd2.2	0.006	0.005	0	2E-04	2E-04	3E-04	1E-03	7E-04	4E-04	5E-05
txnrd3	0.057	0.062	0.018	0.019	0.007	0.016	0.026	0.02	0.016	0.009

Figure 3. Heat map for transcriptions in 7- and 16-month old group. Transcription levels were classified as very low (0.05-fold or lower than the control), low (between 0.05 and 0.2-fold from the control) and high level (higher than 0.2-fold from control). Each transcription level is represented by a different color. Simple averages of 10 replicates were used for the heat-map.

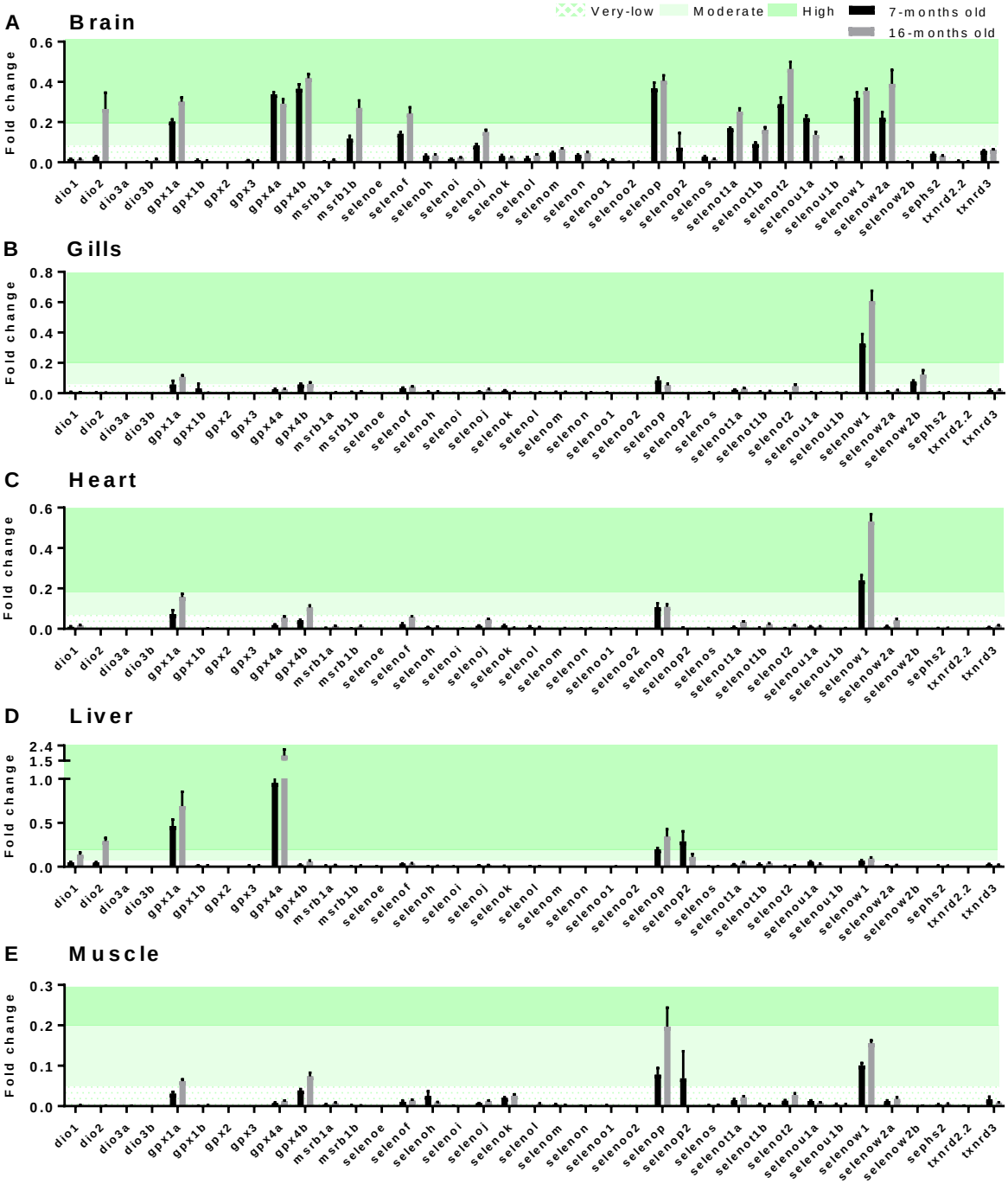


Figure 4. Tissues relative transcription of selenoprotein genes in 7-month-old **(black)** and 16-month-old **(gray)** zebrafish. Data were normalized by the target gene size and RNA-Seq experiment size. Transcription levels are expressed in relative fold changes from an average of *uba52* and *rlp13a* transcription in brain (A), gill (B), heart (C), liver (D) and muscle (E). Results are expressed as average of 10 replicates ± mean standard error. Color indicates

categories of very low level (0.05-fold or lower than the control), low level (between 0.05 and 0.2-fold from the control) and high level (higher than 0.2-fold from control).

Brain

We found 36 selenoprotein-coding gene transcripts in both 7- and 16-months old zebrafish brain at different transcriptional levels (Figure 4A). Only *selenoe,* a chaperone-like-coding gene, and *selenop2* were not transcribed at any group. Both *dio3a* and *gpx2* were not transcribed in fishes at 7-month stage, whereas *selenop2* and *selenow2b* were not transcribed in fishes at 16-month.

In 7-months old we found 19 selenoprotein-coding genes transcribed at very low (*selenow2b, dio3b, selenoo2, selenou1b, msrb1a, txnrd2, gpx3, gpx1b, selenoo1, dio1, selenoi, selenol, selenos, dio2, selenok, selenoh, selenon, sephs2,* and *selenom*), six at low (*txnrd3, selenoj, selenot1b, msrb1b, selenof,* and *selenot1a*) and eight at high transcriptional level (*gpx1a, selenou1a, selenow2a, selenot2, selenow1, gpx4a, gpx4b,* and *selenop*).

In 16-months old group, 18 selenoprotein-coding genes were found to be transcribed at very low level (*dio3a, gpx2, selenoo2, txnrd2.2, gpx3, gpx1b, msrb1a, dio3b, selenoo1, selenos, dio1, selenou1b, selenoi, selenok, sephs2, selenoh, selenol,* and *selenon*), five at low level (*txnrd3, selenom, selenou1a, selenoj,* and *selenot1b*), and 11 at high transcriptional level (*selenof, selenot1a, dio2, msrb1b, gpx4a, gpx1a, selenow1, selenow2a, selenop, gpx4b,* and *selenot2*). Interestingly, *selenop* presented the highest transcriptional level in 7-month-old fishes with 0.37-fold when compared to the mRNA control, whereas the highest transcribed gene in 16-months old fishes was found to be *selenot2* with 0.46-fold when compared to the mRNA control. The main difference between 7- and 16-months old fishes was observed in *dio2* that had an increase of 9-fold (from 0.03 to 0.27-fold).

Gills

The 37 selenoprotein-expressing genes were found to be transcribed in adult fish gills, besides the transcription varied in presence and levels depending on the life stage (Figure 4B). While *gpx2, txnrd2,* and *selenop2* transcripts seemed to be not transcribed in 7-months old fishes, *dio3b* and *dio3a* transcripts were lacked in 16-month fish.

In 7-months old group, we found 29 genes transcribed at very low level (*selenoe, selenoo2, gpx3, dio3b, dio3a, selenoi, selenou1b, msrb1a, selenon, selenol, selenos, dio2, selenoo1, sephs2,*

selenou1a, dio1, msrb1b, selenom, selenot2, selenow2a, selenot1b, selenoh, selenoj, selenok, txnrd3, selenot1a, gpx4a, gpx1b, and *selenof*), four at low level (*gpx1a, gpx4b, selenow2b,* and *selenop*), and only one at high transcriptional level level (*selenow1*).

In 16-months old group, 30 genes were transcribed at very low level (*selenoo2, selenoe, selenop2, txnrd2, gpx3, gpx2, selenoi, dio2, selenoo1, selenou1b, gpx1b, selenos, selenol, selenon, msrb1a, selenou1a, dio1, sephs2, selenok, selenom, msrb1b, selenot1b, selenoh, selenow2a, txnrd3, gpx4a, selenoj, selenot1a, selenof, and selenot2*), four at low level (*selenop, gpx4b, gpx1a,* and *selenow2b*) and none at high transcriptional level. Similar to that observed for 7-months old fish, *selenow1* presented a high level of transcription when compared to the control group (0.6-fold), and its transcriptional rate increased 1.81-fold from 7- to 16-months old fishes (0.33 to 0.6-fold).

Heart

For the dissected heart tissue, we found 35 selenoprotein-coding genes transcribed in adult zebrafish considering the two analyzed life stages (Figure 4C). Only *gpx2* and *selenoe* were not transcribed at all. *Selenow2b* were not transcribed at 7-months old stage and *selenoo2, dio3a, dio2, dio3b, gpx3,* and *selenop2* were found to be not transcribed at 16-months old.

In 7-months old group, we found 30 genes with very low level of transcription level (*selenoo2, dio3a, dio2, selenou1b, dio3b, txnrd2, gpx3, selenoi, gpx1b, selenoo1, selenom, selenon, msrb1b, selenot2, selenos, sephs2, msrb1a, selenop2, selenot1b, selenol, txnrd3, selenoh, selenot1a, dio1, selenou1a, selenow2a, selenoj, selenok, gpx4a, selenof,* and *gpx4b*), two with low level (*gpx1a* and *selenop*) and only one (*selenow1*) with high levels of transcription. In 16-months old group, 23 genes were found to be transcribed at very low level (*txnrd2, selenow2b, gpx1b, selenos, selenou1b, selenoi, selenoo1, selenom, selenon, sephs2, selenok, selenol, selenoh, selenou1a, msrb1b, msrb1a, selenot2, txnrd3, dio1, selenot1b, selenot1a, selenow2a, and selenoj*), five at low level (*gpx4a, selenof, gpx4b, selenop,* and *gpx1a*), and only one at high transcriptional level (*selenow1*).

Interestingly, *selenow1* was found to be highly transcribed in heart tissue for both 7- and 16-months old fishes with 0.24-fold and 0.53-fold, respectively; in a similar fashion as that observed for gills.

Liver

For the dissected liver tissue, we found 35 selenoprotein-expressing genes transcribed in adult zebrafish considering the two analyzed life stages (Figure 4D). Neither *dio3a* nor *gpx2* were transcribed at all, whereas transcription of *dio3b* was lacked only in 16-months old fishes.

In 7-months old group, we found 28 genes with very low level (*dio3b, selenow2b, selenou1b, selenoo2, selenom, txnrd2, selenon, selenoe, selenoi, selenoo1, selenol, selenoh, msrb1b, selenos, selenok, selenot2, msrb1a, sephs2, gpx3, selenoj, gpx1b, selenow2a, gpx4b, selenot1a, txnrd3, selenot1b, selenof,* and *dio2*), four with low level (*dio1, selenou1a, selenow1,* and *selenop*), and four with high levels of transcription (*selenop2, gpx1a,* and *gpx4a*).

In 16-months old group, 26 genes were found to be transcribed at very low level (*selenoo2, selenow2b, selenou1b, selenoe, selenon, selenom, txnrd2, selenoi, selenol, selenoo1, selenok, selenos, selenoh, msrb1b, sephs2, gpx1b, selenot2, gpx3, selenow2a, msrb1a, selenoj, txnrd3, selenou1a, selenof, selenot1b,* and *selenot1a*), four at low level (*gpx4b, selenow1, selenop2,* and *dio1*), and four at high level of transcription (*dio2, selenop, gpx1a,* and *gpx4a*). The highly transcribed gene in liver was found to be *gpx4a* with 1.8-fold higher when compared to control gene mRNA.

Skeletal muscle

In dissected skeletal muscles we found 35 selenoprotein-coding gene transcripts considering both 7- and 16-months-old zebrafishes (Figure 4E). Neither *dio3b* nor *gpx2* were transcribed. Transcripts of both *gpx3* and *selenow2b* were lacked in 7-months old fish tissue, whereas transcripts of *selenoe* was lacked in 16-months.

In 7-months old group-derived muscle tissue, we found 30 genes transcribed at very low level (*selenoo2, dio3a, dio2, selenoe, txnrd2, selenoi, dio1, gpx1b, selenon, selenol, msrb1b, selenoo1, selenos, selenom, msrb1a, selenou1b, sephs2, selenot1b, gpx4a, selenoj, selenof, selenow2a, selenou1a, selenot2, selenot1a, txnrd3, selenok, selenoh, gpx1a,* and *gpx4b*), two genes at low level (*selenop* and *selenow1*), and no gene at high levels of transcription.

In 16-months old group-derived muscle tissue, 29 genes were found to be transcribed at very low level (*selenoo2, txnrd2, gpx3, selenoi, selenow2b, dio2, dio3a, selenon, msrb1b, gpx1b, dio1, selenos, selenom, selenoo1, selenot1b, selenou1b, sephs2, selenol, msrb1a, selenou1a, txnrd3, selenoh, gpx4a, selenoj, selenof, selenow2a, selenot1a, selenok,* and *selenot2*. Low transcript levels were found for four genes (*gpx1a, gpx4b, selenow1,* and *selenop*). No gene exhibited a high

transcription pattern in muscle of 16-month fish. The highest transcribed gene was found to be *selenop* (0.2-fold from control group), presenting from 7- to 16-month an increase from 0.07 to 0.2-fold.

The sum of all transcripts in relation to control for each tissue is presented in the Figure 5A; and the contribution of each gene for selenoprotein diversity in the tissues is represented in Figure 5B. Taken together, the results show that brain of adult zebrafish contains greater number and diversity of selenoproteins compared to other tissues (Figure 5A and 5B). Although the relative level of transcripts had been elevated in liver, it is represented mainly by *gpx4a* and *gpx1a* genes and the diversity of selenoproteins was lower compared to the brain (Figure 5B).

The selenoprotein diversity profile has not changed so much among the ages in most of tissues; however, it is interesting to note the prevalence of *selenop* at 16-months old in relation to the 7-months, where it had smaller and similar transcription levels as *selenop2* (Figure 5B).

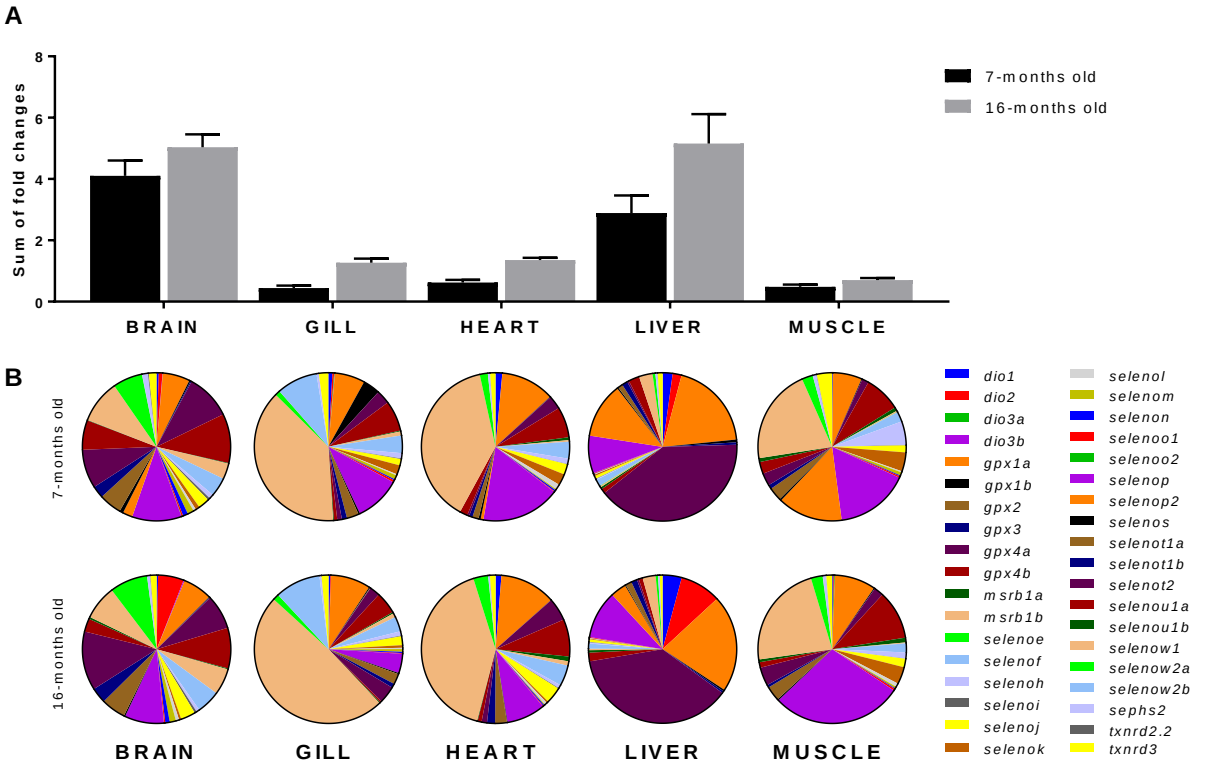


Figure 5. Sum of relative selenoprotein transcript levels among the tissues in 7- (black) and 16-month-old (gray) zebrafish. **(A)** Results from previous figures are summarized here and represent the sum of relative selenoprotein transcription levels from an average of *uba52* and *rlp13a* transcription in brain, gill, heart, liver and muscle. Results are expressed as average of 10 sums ± mean standard error. (B) Graphs representing the tissue selenoprotein

diversity and the individual gene contribution for the total sum of transcripts. The colors are coded by the legend and distributed clockwise.

3.4 Pattern of brain selenoprotein transcription among the ages

Since brain exhibited the highest and more diverse content of transcribed selenoprotein-coding genes, we chose this organ to ascertain the influence of age on selenoprotein gene transcriptional status.

There is no statistical difference in the transcript levels of genes *dio3a, dio3b, gpx1b, gpx2, gpx3, msrb1a, selenoe, selenoh, selenoi, selenok, selenol, selenop2, selenos, selenow1, selenow2b, sephs2,* or *txnrd2.2* among the ages (Supplemental Figure 3). On the order hand, we found 20 selenoprotein genes were differently transcribed through ages, all of them with higher transcript levels in adult phase (*dio1, dio2, gpx1a, gpx4a, gpx4b, msrb1b, selenof, selenoj, selenom, selenon, selenoo1, selenoo2, selenop, selenot1a, selenot1b, selenot2, selenou1a, seleno1b, selenow2a*, and *txnrd3*), having a peak of upregulation mainly at 16-month old (Figure 6).

Except for *selenoo1*, which had more transcription at 39-month old, similar middle transcript levels was found in 7- and 39-month groups.

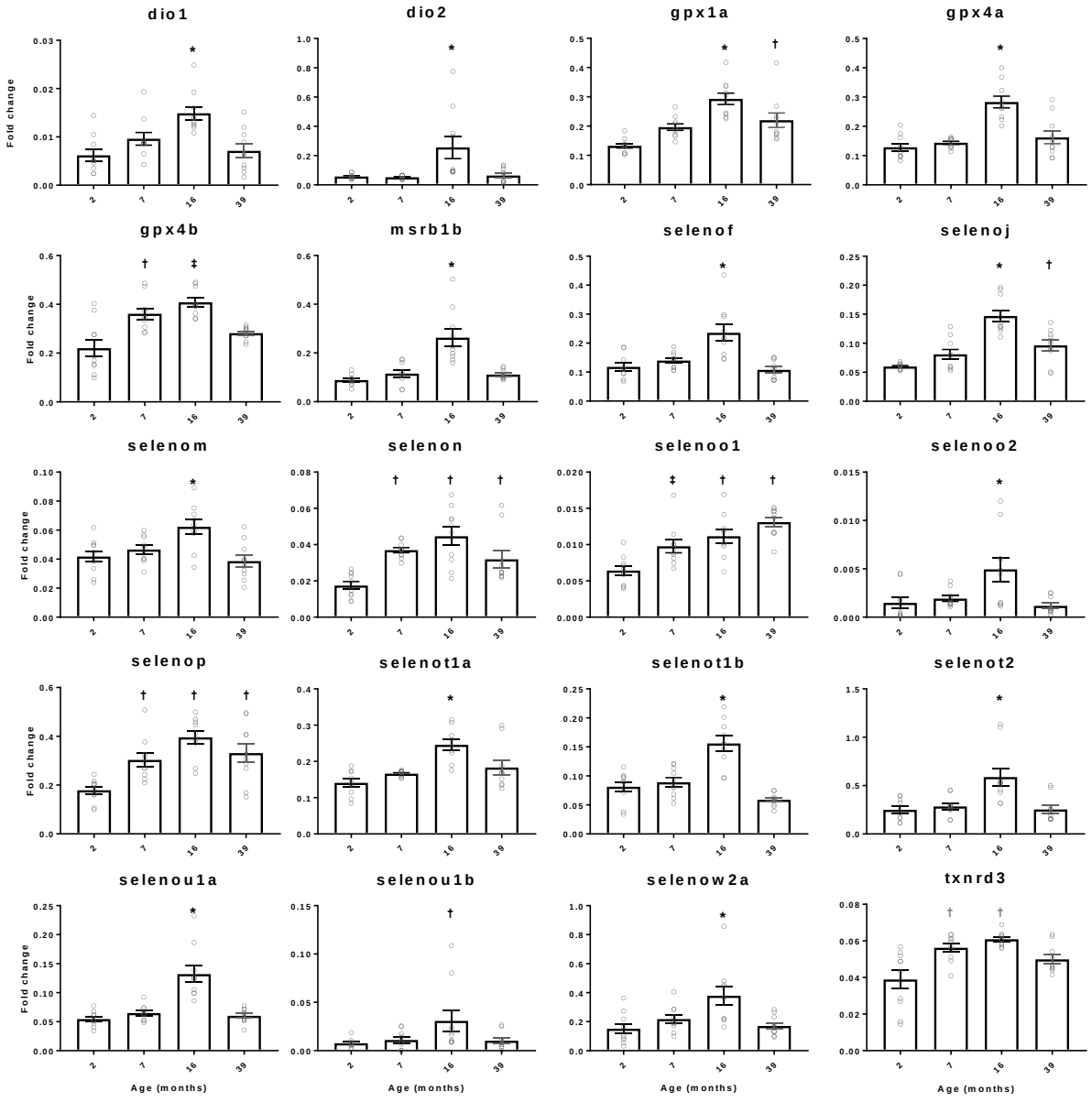


Figure 6. Transcription of selenoprotein genes in zebrafish brain during the different life stages that were significantly altered during aging. Gene transcription was quantified by number of read nucleotide matches with each selenoprotein gene CDS. Data were normalized by the target gene size and RNA-Seq experiment size. Transcription levels are expressed in relative fold changes to an average of *uba52* and *rlp13a* transcription. Results are expressed as average of 10 replicates ± mean standard error and analyzed by one-way ANOVA followed by Tukey multiple comparison test. * indicates significant difference from all other groups, † indicates significant difference from 2-month group, and ‡ indicates significant difference from 2- and 39-month groups.

4 DISCUSSION

In order to denote zebrafish as a powerful organism for translational studies on selenium and selenoproteins, this study described, for the first time, the transcription profile of all 37 selenoproteins from specie. Zebrafish transcriptome data available in literature helped us compare the transcript levels of zebrafish selenoproteins through different life stages and tissues. We found that most queried genes vary in transcription accordingly to age, and tissue. Brain highlighted as tissue with the largest number and diversity of selenoproteins. Zebrafish is the vertebrate with most selenoproteins identified to date, and from these results adult brain can be indicated as an optimal target for studying selenium and selenoprotein modulation.

Zebrafish genome shares approximately 70% identity with human genome. The genome is distributed in 25 autosomal chromosomes with regular centromeres and telomers. It has no sexual chromosome since zebrafish sexual determination is influenced by chromosomic regions (HOWE et al., 2013). Firstly, we localized each selenoprotein-coding gene in the zebrafish genome map, and then confirmed the selenoprotein genes by searching for SECIS sequences. We found an extra SECIS element in the *dio1* gene, and no sequence matched a SECIS element in *selenoj* gene. These results are in conflict with Castellano et al. (2005), that described *selenoj* and reported SECIS region in zebrafish and other fish. They utilized SECISearch 1.0; however, the sequence found was not published or annotated in the genome. SECIS localization within the transcript was also assessed.

Regarding to the specific selenoproteins, there are those with well-known functions as DIO, GPX, and TXNRD families as well as MSRB1B, SELENOP, and SEPHS2; more recent categorized families as SEP15/selenoprotein M and Thioredoxin-like family; and protein families with less characterized functions, including SELENOL, SELENON, SELENOO, SELENOK, SELENOS, SELENOI, SELENOU, and SELENOJ.

GPX represent the largest family in vertebrates. It is the only family to present two conserved gene duplications in zebrafish, with two isoforms for GPX1 and GPX4 (MARIOTTI et al., 2012). Proteins from this family exhibit antioxidant function, detoxifying inorganic and organic peroxides. Although GPX activity is an oxidative stress marker used in various studies (BETANCOR et al, 2015), comparison among isoform expression are scarce. In this study, we found no transcripts for *gpx2* and only discrete levels of *gpx3*. This finding is consistent with mammal counterpart

localization in gastrointestinal tract and plasma, respectively (MOGHADASZADEH et al., 2006). Erickson et al. (2015) reported *gpx2* expression in zebrafish embryo. According to previous works (ZHENG et al., 2013), *gpx4a* in liver samples was the only gene to surpass the control levels of transcription. *gpx1a* was also abundant in most tissues, while *gpx4b* had a strong presence only in brain. Mendieta-Serrano et al. (2015) found zebrafish *gpx4b* shares more sequence similarities with mammal *gpx4* than zebrafish *gpx4a*. Also, *gpx1b* shares synteny similarities with mammal *gpx1*, while *gpx1a* appears be derived from duplication of the ancestral gene. Among different zebrafish life stages, we found all GPX transcription with higher transcription levels in the 16-month old group.

Iodothyronine Deiodinases (DIO), are selenoproteins involved in thyroid hormone production in vertebrates. Thyroid secretes pro-hormone 3,5,3',5'-tyroxine (T4). DIO2 activates T4 to 3,5,3'-triiodothyronine (T3). DIO3 inactivates T4 and T3 removing inner iodine rings. DIO1 catalyzes deiodinase reactions on outer rings (MORENO et al., 1994; BIANCO & LARSEN, 2006). In zebrafish, *dio3b* present similar function to mammal DIO3, whereas *dio3a* codifies a fish-specific protein capable of deiodinating reverse-T3 outer ring (GUO et al., 2014). Houbrechts et al. (2016) reported *dio3a* presence in heart and liver of adult zebrafish. In contrast, herein we did not find high *dio3a* and *dio3b* transcripts, suggesting higher importance of them to earlier life stages since both isoforms are required for zebrafish embryo and larvae development (HEIJLEN, 2014). *dio1* and *dio2* presented a high transcription in 16-month old zebrafish brain.

The Thioredoxin Reductases (TXNRD) are flavoenzymes that reduce thioredoxins and other substrates, with critical role in redox homeostasis (MOGHADASZADEH et al., 2006). While zebrafish do not present a gene ortholog to mammal TXNRD1, *txnrd2.2* code for a protein with high identity to mammal TXNRD2 and *txnrd3* is ortholog to testis localized TXNRD3. Here, genes for TXNRD proteins did not presented high transcription levels in any tissue, but *txnrd3* had high transcription levels at 7- and 16-month old brain.

Methionine-R-Sulfoxide Reductases B1 (MSRB1) are repair enzymes with action against oxidative damage, catalyzing reduction of methionine-R-sulfoxide to methionine (MOGHADASZADEH et al., 2006). Zebrafish have two isoforms of MSRB1, with *msrb1a* presenting higher identity to the ancestral gene than *msrb1b*. In our study, only brain *msrb1b* had high transcripts levels, with a peak at 16-month-old.

Selenophosphate Syntethase 2 (SEPHS2) is a catalyzing enzyme for mono selenium phosphate (MSP) production from a selenite and ATP. It is involved in Sec biosynthesis because MSP is the only Se donor for selenoprotein production. This enzyme is common to all vertebrates (O'LEARY et al., 2016). In zebrafish, we found low levels of *sephs2* transcripts in all tissues, with no difference in brain transcription throughout life stages.

Selenoprotein P (SELENOP) is the protein with more Sec, with 10 Sec residues in humans. It is ancestral to all vertebrates and is responsible for distribution of Se through tissues, with abundance in plasma (BURK, 2010). Zebrafish have two homologs to the ancestral gene, *selenop1* gene has 17 Sec residues and a second SECIS element at 3'-UTR to optimize Sec insertion. Meanwhile, *selenop2* lost the larger N-terminal domain with 16 Sec, maintaining homology with ancestral C-terminal domain, where only one Sec is conserved (KRYUKOV et al., 2000). In our study, we show high transcription of *selenop1* in liver, as well as abundance in other tissues. *selenop2* had significant transcription levels only in liver. High SELENOP presence in zebrafish liver was previously reported (BETANCOR et al., 2015). Although *selenop1* brain transcription has been statistically higher in adult phase, it did not differ between 7-, 16-, and 39-month old zebrafish.

SEP15/selenoprotein M family is a group of proteins with similar structure and thiol-disulfide oxidoreductase predicted function (FERGUSON et al., 2006). In zebrafish, this family is represented by Selenoprotein F (SELENOF), Selenoprotein E (SELENOE), and Selenoprotein M (SELENOM). SELENOF is known to interact with UDP-glucose:glucoprotein glucosyltransferase, playing a role in protein folding (VYACHESLAV et al., 2009). SELENOE probably evolved from SELENOF coding gene and is exclusive to teleosts, but its function is not clear (NOVOSELOV et al., 2006). In our study, *selenoe* transcription was null in all groups analyzed while *selenof* had a marked presence in all tissues. SELENOM is present in all vertebrates and seems has a role in neuroprotection and cytosolic calcium regulation, but its function still not well elucidated (REEVES et al., 2010). Corroborating with the suggested role in neuroprotection, the largest *selenom* transcription found in our study was in zebrafish brain. Both *selenof* and *selenom* presented higher levels of transcription in the 16-month-old zebrafish brain.

Thioredoxin-like (Rdx) family is a group of proteins with conserved Rdx fold. Rdx folds are two cysteines separated by two amino acids (CxxC), selenoproteins from this family substituted a cysteine for a selenocysteine, forming a CxxU motif (DIKIY et al., 2007). In zebrafish, the family

is represented by Selenoprotein H (SELENOH), and three forms of both Selenoprotein T (SELENOT) and Selenoprotein W (SELENOW). SELENOH is known to protect DNA damage and plays a role in redox homeostasis (COX et al., 2016) and zebrafish development (AMSTERDAM et al., 2004). In our study, we found very low transcript levels of *selenoh* in all tissues, that also did not vary in brain through the life stages analyzed. SELENOT is known to has cellular adhesion function (SENGUPTA et al., 2019) and the ancestral *selenot* gene in zebrafish was duplicated in two different occasions. Herein, *selenot1a*, *selenot1b*, and *selenot2* were highly transcribed in zebrafish brain when compared with to the other tissues, having a similar transcription pattern throughout ages, with a higher peak in 16-month old zebrafish brain. SELENOW is a group of proteins with GSH-dependent antioxidant role (NOH et al., 2010). SELENOW1 and SELENOW2 are ancestral proteins to all vertebrates. While a second gene for SELENOW2 originated in fish, terrestrial vertebrates lost him in evolution. In our results, we found *selenow1* to be the most transcripted selenoprotein in gill, heart and muscle tissues, representing between 20 and 53% of total selenoprotein transcription. The duplicates *selenow2a* and *selenow2b* differed drastically in our results. The first was present in all tissues while the later had a significative presence only in zebrafish gill. Also, *selenow2a* was the only to show difference in transcription by aging, with significantly higher presence in 16-month-old zebrafish brain.

Selenoprotein L (SELENOL) is classified within thioredoxin superfamily and is exclusive to fish. It is the only selenoprotein that uses two Sec residues in a motif UxxU (SHCHEDRINA et al., 2007). Selenoprotein N (SELENON) is a protein important to cell protection against oxidative stress and zebrafish muscle development and differentiation (JURYNEC et al., 2008). We found that *selenol* and *selenon* have very low transcription in the samples analyzed, with the highest presence in the brain. *selenon* has a significative increase in 7-, 16-, and 39-month fish brain. Selenoprotein O (SELENOO) is a protein with redox activity common to all vertebrates (HAN et al., 2014), with a gene duplication in zebrafish lineage. Dudkiewicz et al. (2012) suggested that the SELENOO function might be linked to ABC transport regulation. Our data showed a very low transcription of both *selenoo1* and *selenoo2* in all samples analyzed. We found a difference in relation to the duplicate responses to aging, where the *selenoo1* had a lower brain transcription in the 2-month-old group and selenoo2 higher levels at 16-month-old group.

Selenoprotein K (SELENOK) is a transmembrane protein involved in endoplasmic reticulum protein folding (chaperone-like). It has a role in cell protection against apoptosis and is important

to protein palmitoylation (SHCHEDRINA et al., 2011; LEE et al., 2015; FREDERICKS et al., 2015). Selenoprotein S (SELENOS) is involved in protein degradation in plasmatic reticulum and might also be involved in inflammation control (MOGHADASZADEH et al., 2006). Selenoprotein I (SELENOI) is a protein predicted to be involved with lipid synthesis and protein folding (HENNEBERRY et al., 1999). In the present work, *selenok selenos* and *selenoi* showed very low transcription in the tissues.

Selenoprotein U (SELENOU) is a protein predicted to be involved in redox processes with homology to human peroxiredoxin-like 2A (PRXL2A) (CASTELLANO et al., 2005). In our study, both duplicates of the gene were more expressed in zebrafish brain rather than other tissues, with a higher presence of selenou1a than selenou1b. Both duplicates also had a peak in transcription at 16-month old brain.

Selenoprotein J (SELENOJ) is a protein ancestral to all vertebrates, but nowadays conserved only in fish. It is preferentially expressed in embryo optical lens and shares similarities to crystallin proteins (CASTELLANO et al., 2005). Its structural role is unique among the selenoproteins studied here and the role of a Sec residue is unknown. We found very low *selenoj* transcription but it had a significative increase in 16-month-old zebrafish brain. Searching for SECIS sequences, we found no match for a proper sequence using the available tools.

Recently, five new proteins were indicated as first potential selenoproteins translated with no SECIS element present in the transcript (GUO et al., 2018). Among the candidates, zebrafish possesses genes that code proteins homolog to FXYD2, ATP5B, and MT2; but no homologs for SCGB1A1 or MUP. Studies with these proteins might highlight other ways for which the organisms incorporate Se into proteins.

The lack of determination of neither sex distribution nor zebrafish strain utilized by the original research group was a limitation for our study. Previous works have shown the importance of both factors on zebrafish selenoprotein expression and Se status alterations (BENNER et al., 2010; DREW et al., 2012). Additionally, we made a choice to study this set of transcriptome data which had different age and tissues groups in the same laboratorial conditions, but there are transcriptome data encompassing more life stages – like embryonic phase – and/or other relevant tissues. In this sense, in a further work we intend to broad the knowledge of zebrafish selenoprotein transcription profile. In fact, only with broader studies we can ascertain whether genes with no transcription found in the groups analyzed here are considered non-functionals or just specifically localized in

the organism/life stage. Indeed, SECIS prediction absence for *selenoj* is intriguing and more studies must be made to clarify the specific region that coordinates Sec insertion in the transcript.

Under an evolutionary perspective, Lobanov et al. (2007) postulated that aquatic organisms maintain a higher number of selenoproteins than their terrestrial counterparts due to the availability of selenium in the environment. It is yet to be determined whether this influence can also explain the larger selenoproteomes in fish compared with terrestrial vertebrates, since vertebrates are less affected by environmental conditions. From our results, we suppose that zebrafish has maintained most of its duplicated selenoprotein gene active, suggesting the environment is not leading to loss of Se use.

Collectively, the data from the present study support zebrafish and especially adult brain as potent platforms for studies toward selenium and selenoproteins.

5 REFERENCES

1. AMSTERDAM, A.; NISSEN, R. M.; SUN, Z. et al. Identification of 315 genes essential for early zebrafish development. **Proc Natl Acad Sci** 101:12792-12797. 2004.
2. BARBAZUK, W. B.; KORF, I.; KADAVI, C. et al. The syntenic relationship of the zebrafish and human genomes. **Genome Res** 1351–1358. 2000.
3. BENNER, M. J.; DREW, R. E.; HARDY, R. W., et al. Robison BD. Zebrafish (*Danio rerio*) vary by strain and sex in their behavioral and transcriptional responses to selenium supplementation. **Comp Biochem Physiol A Mol Integr Physiol** 157:310-8. 2010.
4. BENNER, M. J.; SETTLES, M. L.; MURDOCH, G. K. et al. Sex-specific transcriptional responses of the zebrafish (*Danio rerio*) brain selenoproteome to acute sodium selenite supplementation. **Physiol Genomics** 45:653-66. 2013.
5. BETANCOR, M. B.; ALMAIDA-PAGÁN, P. F.; SPRAGUE M. et al. Roles of selenoprotein antioxidant protection in zebrafish, *Danio rerio*, subjected to dietary oxidative stress. **Fish Physiol Biochem** 41:705-20. 2015.
6. BIANCO, A. C., LARSEN, P. R. Selenium, deiodinases and endocrine function. In: Hatfield DL, Berry MJ, Gladyshev VN, editors. Selenium: Its molecular biology and role in human health. **Springer**. pp. 207–219. 2006.
7. BIFANO, A.L., T. ATASSI, T. FERRARA and D. M. Driscoll, Identification of nucleotides and amino acids that mediate the interaction between ribosomal protein L30 and the SECIS element. **BMC Mol. Biol**., 2013, 14, 12
8. BÖSL, M.R.; TAKAKU, K.; OSHIMA, M.; Nishimura, S. Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp). **Proc. Natl. Acad. Sci**. 94:5532–5534. 1997.
9. BUDIMAN, M. E.; BUBENIK, J. L.; MINIARD, A. C. et al. Eukaryotic Initiation Factor 4a3 Is a Selenium-Regulated RNA-Binding Protein that Selectively Inhibits Selenocysteine Incorporation. **Mol. Cell** 35:479–489. 2009.
10. BUETTNER, C., HARNEY, J. W., BERRY, M. J. The *Caenorhabditis elegans* Homologue of Thioredoxin Reductase Contains a Selenocysteine Insertion Sequence (SECIS) Element That Differs from Mammalian SECIS Elements but Directs Selenocysteine Incorporation. **Journal of Biological Chemistry**, 274:21598–21602. 1999.

11. BURK, R. F., & HILL, K. E. Selenoprotein P-expression, functions, and roles in mammals. **Biochimica et biophysica acta**, 1790(11), 1441–1447. 2009
12. BURK, R. F.; HILL, K. E. Regulation of Selenium Metabolism and Transport. **Annu. Rev. Nutr**.35:109-134. 2015.
13. CAO, J., NAVIS, A., COX, B. D., et al. Single epicardial cell transcriptome sequencing identifies Caveolin-1 as an essential factor in zebrafish heart regeneration. **Development** 143(2):232-43. 2016.
14. CARDOSO, B. R.; ROBERTS, B. R.; BUSH, A. I. et al. Selenium, selenoproteins and neurodegenerative diseases. **Metallomics** 7:1213-1228. 2015.
15. CASTELLANO, S.; ANDRES, A. M.; BOSCH, E., et al. Low exchangeability of selenocysteine, the 21st amino acid, in vertebrate proteins. **Mol Biol Evol** 26:2031-2040. 2009.
16. CASTELLANO, S.; LOBANOV, A.V.; Chapple, C. et al. Diversity and functional plasticity of eukaryotic selenoproteins: Identification and characterization of the SelJ family. **Proc Natl Acad Sci** 102(45):16188-16193. 2005.
17. CASTELLANO, S.; MOROZOVA, N.; MOREY, M. et al. In silico identification of novel selenoproteins in the Drosophila melanogaster genome. **EMBO Rep** 2:697-702. 2001
18. CHAPPLE, C. E.; GUIGO, R. Relaxation of selective constraints causes independent selenoprotein extinction in insect genomes. **PLoS One** 3:e2968. 2008.
19. CONE, J. E., DEL RÍO, R. M., DAVIS, J. N., et al. Chemical characterization of the selenoprotein component of clostridial glycine reductase: identification of selenocysteine as the organoselenium moiety. **Proc. Natl. Acad. Sci**. U. S. A. 73, 2659−2663. 1976.
20. COX, A.G., TSOMIDES, A., KIM, A.J., et al. Selenoprotein H is an essential regulator of redox homeostasis that cooperates with p53 in development and tumorigenesis. **Proc. Natl. Acad. Sci**. USA. 113:5562-71. 2016.
21. DOLGOVA, N.V.; NEHZATI, S.; MACDONALD, T. C. et al. Disruption of selenium transport and function is a major contributor to mercury toxicity in zebrafish larvae. **Metallomics**. 11:621-631. 2019.
22. DREW, R.E.; SETTLES, M. L.; CHURCHILL, E. J. et al. Brain transcriptome variation among behaviorally distinct strains of zebrafish (*Danio rerio*). **BMC Genomics**. 13:323. 2012.

23. DUDKIEWICZ, M.; SZCZEPIŃSKA, T.; GRYNBERG, M. et al. A Novel Protein Kinase-Like Domain in a Selenoprotein, Widespread in the Tree of Life. **PLoS ONE** 7(2): e32138. 2012.
24. DUMONT, E.; VANHAECKE, F.; CORNELIS, R. Selenium speciation from food source to metabolites: a critical review. **Anal Bioanal Chem** 385:1304–23. 2006
25. ERICKSON, T.; NICOLSON, T. Identification of sensory hair-cell transcripts by thiouracil-tagging in zebrafish. **BMC Genomics** 16:842. 2015.
26. FAGEGALTIER D.; LESCURE A.; WALCZAK P. et al. Structural analysis of new local features in SECIS RNA hairpins. **Nucleic Acids Res** 28(14):2679-89. 2000
27. FERGUSON, A., LABUNSKYY, V. M., FOMENKO, D. E. et al. NMR Structures of the Selenoproteins Sep15 and SelM Reveal Redox Activity of a New Thioredoxin-like Family. **The Journal of Biological Chemistry** 281, 3536-3543. 2006.
28. FLOHE, L.; GÜNZLER, W.; SCHOCK, H. Glutathione peroxidase: A selenoenzyme **FEBS Lett** 32, 132–134 1973
29. FREDERICKS, G. J.; HOFFMANN, P. R. Selenoprotein K and protein palmitoylation. **Antioxid Redox Signal** 23(10):854-62. 2015.
30. GLADYSHEV, V. N.; ARNÉR, E. S., BERRY, M. J., et al. Selenoprotein Gene Nomenclature. **J Biol Chem**, 291(46), 24036–24040. 2016 .
31. GOBLER, C. J.; LOBANOV, A.V.; TANG, Y.Z. et al. The central role of selenium in the biochemistry and ecology of the harmful pelagophyte, *Aureococcus anophagefferens*. **ISME J** 7: 1333–1343, 2013.
32. GOLDSMITH, P. Zebrafish as a pharmacological tool: The how, why and when. **Curr Opin Pharmacol**, 4:504–512, 2004.
33. GRIM, J. M.; HYNDMAN, K.A.; KRISKA, T. et al. Relationship between oxidizable fatty acid content and level of antioxidant glutathione peroxidases in marine fish. **J Exp Biol**. 214 (22): 3751–3759. 2011.
34. GRUNDNER-CULEMANN, E.; MARTIN, G. W.,3rd; HARNEY, J. W. et al. Two distinct SECIS structures capable of directing selenocysteine incorporation in eukaryotes. **RNA** 5(5), 625–635. 2019.

35. GUO, C., CHEN, X., SONG, H., et al. Intrinsic Expression of a Multiexon Type 3 Deiodinase Gene Controls Zebrafish Embryo Size. **Endocrinology**. 155(10):4069-80. 2014.
36. GUO, L., YANG, W., HUANG, Q., et al. Selenocysteine-Specific Mass Spectrometry Reveals Tissue-Distinct Selenoproteomes and Candidate Selenoproteins. **Cell Chemical Biology**. 2018
37. HAMILTON, S. J. Review of selenium toxicity in the aquatic food chain**. Sci Total Environ** 326:1–31. 2004.
38. HAN, S. et al. "Characterization of mammalian selenoprotein o: a redox-active mitochondrial protein." **PloS one** vol. 9,4 e95518. 2014.
39. HARTIKAINEN, H.L. Biochemistry of selenium and its impact on food chain quality and human health. **J. Trace Elem. Med. Biol**. 18, 309–318. 2015
40. HATFIELD, D.L.; GLADYSHEV, V.N. How selenium has altered our understanding of the genetic code. M**ol. Cell. Biol**. 22, 3565–3576. 2002
41. HEBERLE, H.; MEIRELLES, G. V.; DA SILVA, F. R. et al. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. **BMC Bioinformatics** 16:169. 2015.
42. HEIDEN, T.C.; STRUBLE, C.A.; RISE, M.L., et al. Molecular targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) within the zebrafish ovary: Insights into TCDD-induced endocrine disruption and reproductive toxicity. **Reproductive toxicology** 25:47-57. 2008.
43. HEIJLEN, M.; HOUBRECHTS, A. M.; BAGCI E. A. et al. Knockdown of Type 3 Iodothyronine Deiodinase Severely Perturbs Both Embryonic and Early Larval Development in Zebrafish, **Endocrinology**, 155:1547–1559. 2013
44. HENNEBERRY, A. L.; MCMASTER, C. R. Cloning and expression of a human choline/ethanolaminephosphotransferase: synthesis of phosphatidylcholine and phosphatidylethanolamine. **Biochem J** 339: 291–298. 1999.
45. HOLBEN, D.H.; SMITH, A.M. The diverse role of selenium within selenoproteins: A review. Jo**urnal of the American Dietetic Association** 99:836-843. 1999.
46. HOUSECROFT, C.; SHARPE, A.G. Inorganic chemistry. 4th ed. **Harlow: Pearson Education Limited**; chapter 15. 2012;

47. HOWE, K.; CLARK, M. D.; TORROJA, C. F. et al. The zebrafish reference genome sequence and its relationship to the human genome. **Nature**, 496(7446), 2013.
48. JAPHA, A. Experimental nonnulla de vi selenii in organismum animalen. **Dissertation**, Halle. 1842 (Cit. Moxon & Rhian, 1943.)
49. JUKES T. H. Selenium, an "essential poison". J Appl Biochem 5:233-234. 198
50. JURYNEC, M.J. et al. “Selenoprotein N is required for ryanodine receptor calcium release channel activity in human and zebrafish muscle.” **Proceedings of the National Academy of Sciences of the United States of America** vol. 105,34:12485-90. 2008
51. KATOH, K.; MISAWA, K.; KUMA, K. et al. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. **Nucleic acids research**, 30(14), 3059–3066. 2002.
52. KERVESTIN, S.; JACOBSON, A. NMD: A multifaceted response to premature translational termination. **Nat. Rev. Mol. Cell. Biol**. 13, 700–712. 2012
53. KESHAN DISEASE RESEARCH GROUP OF THE CHINESE ACADEMY OF MEDICAL SCIENCES. Observations on effect of sodium selenite in prevention of Keshan disease. **Chin. Med. J.** 92, 471−476. 1979
54. KIJIMA, Y.; WANTONG, W.; IGARASHI, Y. et al. Age-associated different transcriptome profiling in zebrafish and rat: insight into diversity of vertebrate aging. **bioRxiv** 478438; 2018
55. KOIVISTOINEN, P.; HUTTUNEN, J. K. Selenium in food and nutrition in Finland: An overview on research and action. **Ann. Clin. Res**. 18, 13−17. 1986
56. KRYUKOV GV, et al. Characterization of mammalian selenoproteomes. **Science**, 2003 May 30. PMID 12775843
57. KRYUKOV GV, KRYUKOV VM, GLADYSHEV VN. New mammalian selenocysteine-containing proteins identified with an algorithm that searches for selenocysteine insertion sequence elements. **J Biol Chem** 274:33888-33897. 1999.
58. KRYUKOV, G.V. and GLADYSHEV, V.N. Selenium metabolism in zebrafish: multiplicity of selenoprotein genes and expression of a protein containing 17 selenocysteine residues. **Genes to cells : devoted to molecular & cellular mechanisms**. 5(12):1049-1060. 2000.

59. LABUNSKYY, V. M.; HATFIELD, D. L.; Gladyshev, V. N. Selenoproteins: molecular pathways and physiological roles. **Physiol. Rev**., 94:739–777. 2014.
60. LABUNSKYY, V. M.; YOO, M.; HATFIELD, D. L., et al. Sep15, a Thioredoxin-like Selenoprotein, Is Involved in the Unfolded Protein Response and Differentially Regulated by Adaptive and Acute ER Stresses. **Biochemistry** 48:8458-8465. 2009.
61. LATRECHE, L.; JEAN-JEAN, O.; DRISCOLL, D.M.; Chavatte, L. Novel structural determinants in human SECIS elements modulate the translational recoding of UGA as selenocysteine. **Nucleic Acids Res**. 37, 5868–5880. 2009
62. LEE, J. H.; PARK, K. J.; JANG, J. K. et al. Selenoprotein S-dependent Selenoprotein K Binding to p97(VCP) Protein Is Essential for Endoplasmic Reticulum-associated Degradation. **The Journal of biological chemistry**, 290(50), 29941–29952. doi:10.1074/jbc.M115.680215. 2015.
63. LEMLY, A.D. Assessing the toxic threat of selenium to fish and aquatic birds. **Environ Monit Assess**.43:19–35 1996.
64. LESCURE, A.; GAUTHERET, D.; CARBON, P. et al. Novel selenoproteins identified in silico and in vivo by using a conserved RNA structural motif. **J Biol Chem** 274:38147-38154. 1999.
65. LIESCHKE, G.J.; CURRIE P.D. Animal models of human disease: zebrafish swim into view. **Nat Rev Genet**, v. 8, p. 353–367, 2007.
66. LOBANOV, A. V.; HATFIELD, D. L.; GLADYSHEV, V. N. Selenoproteinless animals: selenophosphate synthetase SPS1 functions in a pathway unrelated to selenocysteine biosynthesis. **Protein Sci**. 17,129. 176−182. 2008
67. LOBANOV, A.V.; HATFIELD, D.L.; GLADYSHEV, V.N. 2008. Reduced reliance on the trace element selenium during evolution of mammals. **Genome Biol** 9:R62
68. LOW, S.L.; GRUNDNER-CULEMANN, E.; HARNEY, J.W.; Berry, M.J. SECIS-SBP2 interactions dictate selenocysteine incorporation efficiency and selenoprotein hierarchy. **EMBO J**. 2000, 19, 6882–6890
69. MARELLI, F.; CARRA, S.; AGOSTINI, M. et al. Patterns of thyroid hormone receptor expression in zebrafish and generation of a novel model of resistance to thyroid hormone action. **Molecular and Cellular Endocrinology**. 424:102-17. 2016.

70. MARIOTTI, M. A.; LOBANOV, V.; GUIGO, R. et al. SECISearch3 and Seblastian: new tools for prediction of SECIS elements and selenoproteins. **Nucl. Acids Res**. 2013.
71. MARIOTTI, M.; RIDGE, P. G.; ZHANG, Y. et al. Composition and Evolution of the Vertebrate and Mammalian Selenoproteomes. **PLoS ONE** 7(3):e33066. 2012.
72. MARTIN, G. W., 3rd; HARNEY, J. W.; BERRY, M. J. Selenecysteine incorporation in eukaryotes: Insights into mechanism and efficiency from sequence, structure, and spacing proximity studies of the type I deiodinase SECIS element. **RNA** 2, 171–182. 1996.
73. MARTIN-ROMERO, F. J.; KRYUKOV, G. V.; LOBANOV, A. V. et al. Selenium metabolism in *Drosophila*: selenoproteins, selenoprotein mRNA expression, fertility, and mortality. **J Biol Chem** 276:29798-29804 2001.
74. MENDELEV, N. et al. "Upregulation of human selenoprotein H in murine hippocampal neuronal cells promotes mitochondrial biogenesis and functional performance." **Mitochondrion** 11.1 (2011): 76-82.
75. MENDIETA-SERRANO, M. A.; SCHNABEL-PERAZA, D.; LOMELÍ, H. et al. Spatial and temporal expression of zebrafish glutathione peroxidase 4 a and b genes during early embryo development. **Gene Expression Patterns**. 19(1-2):98-107. 2015.
76. MOGHADASZADEH, B.; BEGGS, A. H. "Selenoproteins and their impact on human health through diverse physiological pathways." **Physiology (Bethesda, Md.)** vol. 21 (2006): 307-15.
77. MORENO, M., BERRY, M.J.; HORST, C. et al. Activation and inactivation of thyroid hormone by type I iodothyronine deiodinase.**FEBS Lett**. 1994 May 16; 344(2-3):143-6.
78. MORTAZAVI, A.; WILLIAMS, B. A.; MCCUE, K. et al. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature methods. 2008 Jul 1;5(7):621-8.
79. MOXON A.L.; RHIAN, M. 1943. Selenium poisoning. **Physiol. Reviews** 23(4):305-337. 1943.
80. NOH, O. J., PARK, Y. H., CHUNG, Y. W., et al. Transcriptional regulation of selenoprotein W by MyoD during early skeletal muscle differentiation. **Journal of Biological Chemistry**, 285(52), 40496-40507. 2010.
81. NOVOSELOV, S. V. et al. Identification and characterization of Fep15, a new selenocysteine-containing member of the Sep15 protein family. **The Biochemical journal** 394:575-579. 2006.

82. OLDFIELD, J. E. The selenium story: some reflections on the “moon-metal”. **N. Z. Vet. J.** 22:85−94. 1974.
83. OLIVEIRA, C. S.; PICCOLI, B. C.; ASCHNER, M. et al. Chemical Speciation of Selenium and Mercury as Determinant of Their Neurotoxicity. **Neurotoxicity of Metals**, 53–83. 2017.
84. PAINTER, E. P.; FRANKE, K. W. SELENIUM IN PROTEINS FROM TOXIC FOODSTUFFS: III. THE REMOVAL OF SELENIUM FROM TOXIC PROTEIN HIDROLYSATES J. Biol. Chem. 111:643-651. 1935.
85. PAPP, L. V.; LU, J.; HOLMGREN, A., et al. From Selenium to Selenoproteins: Synthesis, Identity, and Their Role in Human Health. **Antioxidants & Redox Signaling**, 9:775–806. 2007.
86. PENGLASE, S.; HAMRE, K.; ELLINGSEN S. Selenium prevents downregulation of antioxidant selenoprotein genes by methylmercury. **Free Radic Biol Med**. 75:95-104. 2014a
87. PENGLASE, S.; HAMRE, K.; RASINGER, J. D; et al. Selenium status affects selenoprotein expression, reproduction, and F_1 generation locomotor activity in zebrafish (*Danio rerio*). **Br J Nutr** 111:1918-31. 2014b.
88. PINSENT, J. The need for selenite and molybdate in the formation of formate dehydrogenases by members of the *Coliaerogenes* group of bacteria. **Biochem J** 57:10−16. 1954.
89. RAJPUT, B.; PRUITT, K. D.; MURPHY, T. D. RefSeq curation and annotation of stop codon recoding in vertebrates. **Nucleic acids research**, 47:594–606. 2019.
90. REEVES, M.A.; BELLINGER, F. A.; BERRY, M. J. Antioxidants & Redox Signaling.Apr 2010.**ahead of print** http://doi.org/10.1089/ars.2009.2883
91. REICH, H. J.; HONDAL, R. J. Why Nature Chose Selenium. **ACS Chemical Biology**, 11:821–841. 2016.
92. ROBINSON, D. N.; COOLEY, L. Examination of the function of two kelch proteins generated by stop codon suppression. **Development** 124:1405-1417. 1997.
93. ROBINSON, W. O. **J. Assn. Of. Agric. Chem**., 16, 423 1933. (Cit: Painter & Franke, 1935)

94. ROCHA, J. B. T.; PICCOLI, B. C.; OLIVEIRA, C.S. Biological and chemical interest in selenium: a brief historical account. **ARKIVOC** 5550190.p009.784. 2017

95. ROMAGNÉ, F.; SANTESMASSES, D.; WHITE, L. et al. SelenoDB 2.0: annotation of selenoprotein genes in animals and their genetic diversity in humans. **Nucleic Acids Res**, 42:437-443. 2014.

96. ROSENFELD, I.; BEATH, O.A. Selenium Geobotany, Biochemistry, Toxicity and Nutrition. Economic Botany, 20, 340-343. 1964

97. SCHWARZ, K.; FOLTZ, C. M. Selenium as an integral part of factor 3 against dietary liver degeneration. **J Am Chem Soc** 79:3292−3293. 1957

98. SELINUS, O.; ALLOWAY, B.; CENTENO, J. A. et al. Essentials of Medical Geology: Impacts of the Natural Environment on Public Health. **Burlington**: Elsevier Academic Press. 2005.

99. SENGUPTA, A.; CARLSON, B. A.; LABUNSKYY, V. M. et al. Selenoprotein T deficiency alters cell adhesion and elevates selenoprotein W expression in murine fibroblast cells. **Biochemistry and Cell Biology**, 87, 953-961. 2009.

100. SHAMBERGER, R. J. Selenium and health. In Trace elements in health: A review of current issues. **Rose, J., Ed.** 167−181, 1983

101. SHCHEDRINA, V. A.; EVERLEY, R. A.; ZHANG, Y. et al. (2011). Selenoprotein K binds multiprotein complexes and is involved in the regulation of endoplasmic reticulum homeostasis. **The Journal of biological chemistry** 286:42937–42948. 2011.

102. SHIN, J. T.; FISHMAN, M. C. From Zebrafish to human: modular medical models. **Annu Rev Genomics Hum Genet** 3:311–340. 2002.

103. SUNDE, R.A.; RAINES, A.M.; BARNES, K.M.; et al. Selenium status highly regulates selenoprotein mRNA levels for only a subset of the selenoproteins in the selenoproteome. **Biosci Rep** 29:329–338. 2009.

104. TAN, J. A.; HOU, S. F.; ZHU, W. et al. The Keshan disease in China: a study of the geographical epidemiology. **Acta Geographica Sinica** 34:85−104. 1979.

105. TAYLOR, J. S.; BRAASCH, I.; FRICKEY, T. et al. Genome duplication, a trait shared by 22000 species of ray-finned fish. **Genome research** 13:382–390. 2003.

106. TROFAST, J. Berzelius' Discovery of Selenium. **Chemistry International** 33:16−19. 2011.

107. VIHTELIC, T. S.; FADOOL, J. M.; GAO, J.; et al. Expressed sequence tag analysis of zebrafish eye tissues for NEIBank. **Molecular Vision**. 11:1083-1100. 2005.

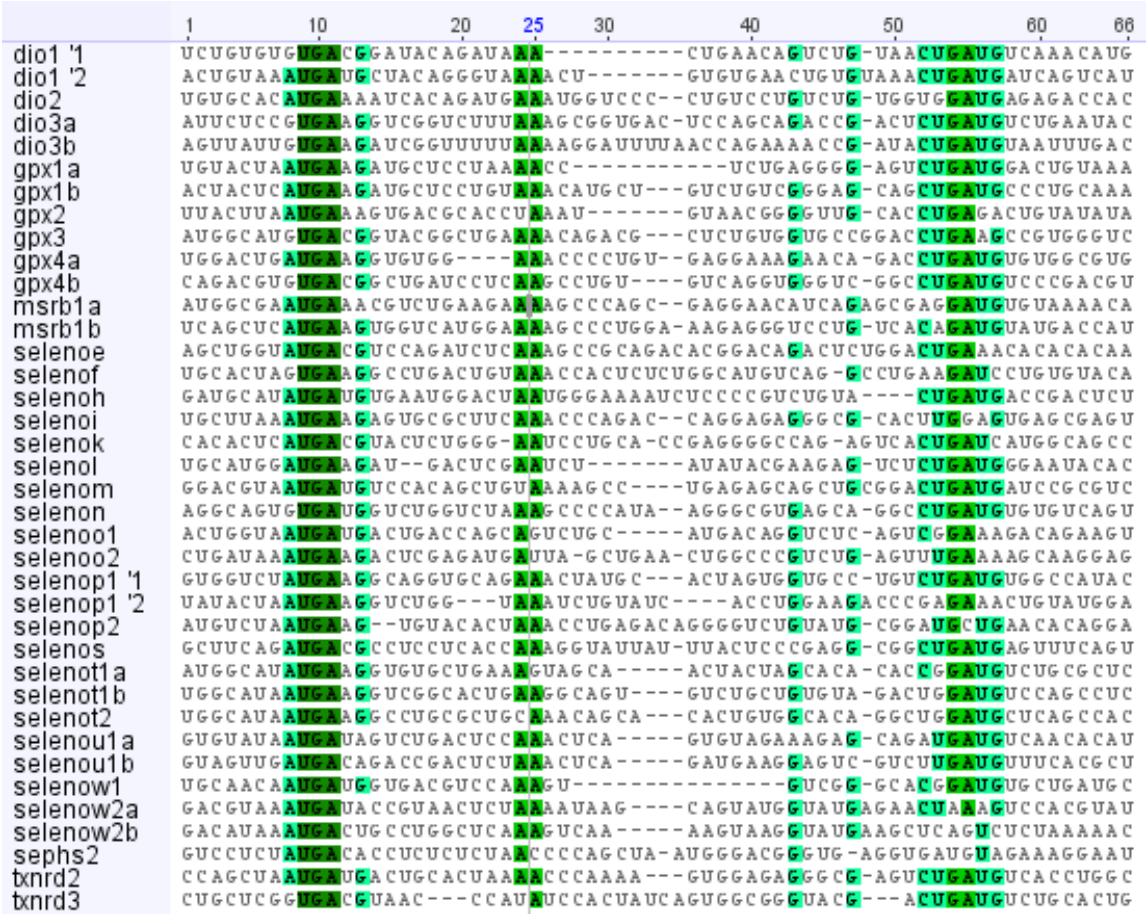
108. WU, R.; SHEN, Q.; NEWBURGER, P.E. Recognition and binding of the human selenocysteine insertion sequence by nucleolin. **J. Cell. Biochem**. 77:507–516. 2000.

109. YAMASHITA, Y.; YABU, T.; TOUHATA, K. et al. Purification and characterization of glutathione peroxidase 1 in the red muscle of Pacific bluefin tuna *Thunnus orientalis*. **Fisheries science** 78:407-413. 2012.

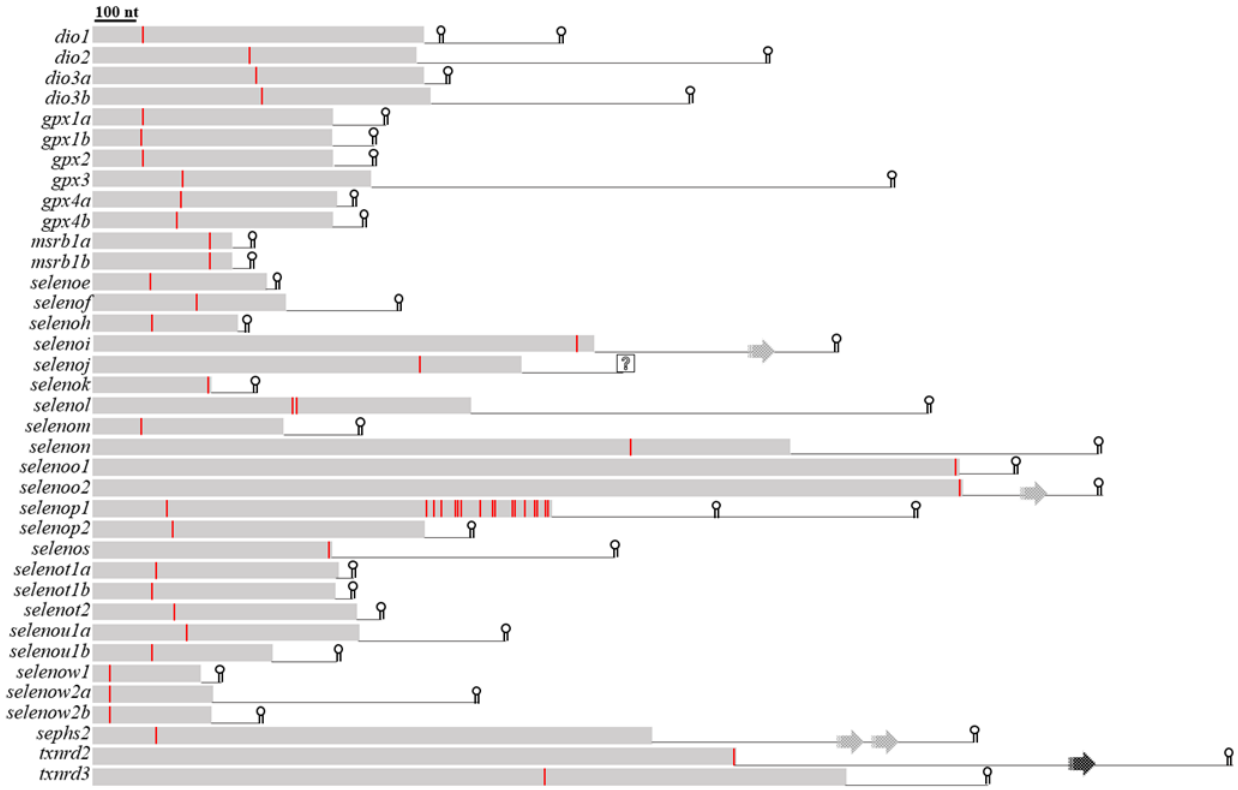
110. YAN, J.; FEI, Y.; HAN, Y. et al. Selenoprotein O deficiencies suppress chondrogenic differentiation of ATDC5 cells. **Cell Biol Int**, 40:1033-1040. 2016.

111. ZHANG, L.; ZHU, J. H.; ZHANG X.; Cheng WH. The Thioredoxin-Like Family of Selenoproteins: Implications in Aging and Age-Related Degeneration. **Biol Trace Elem Res**. 188189-195. 2018.

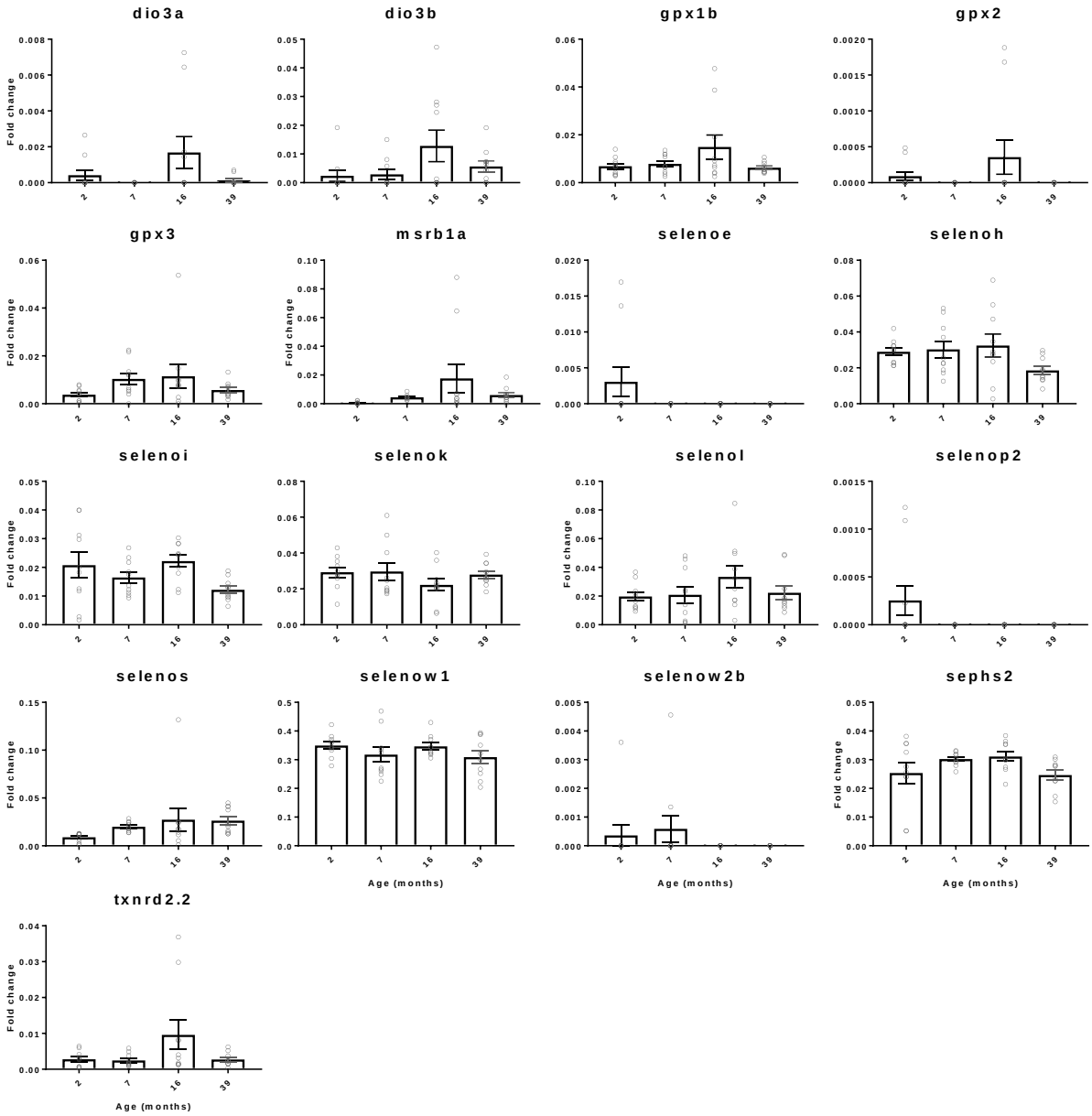
112. ZHENG, W.; XU, H.; LAM, S.H. et al. Transcriptomic analyses of sexual dimorphism of the zebrafish liver and the effect of sex hormones. **PLOS ONE**. 8 (1): e53562. 2013.



Supplemental Figure 1. Alignment of SECIS elements for each zebrafish selenoprotein transcript. Green backgroung indicates identity among conserved base pairs. ‘1 and ‘2 indicate first and second SECIS downstream to the translated region.



Supplemental Figure 2. Characterization of selenoprotein mRNA in zebrafish. Grey bar: nucleotide size, red marker: Sec codon, loop: SECIS element, thin grey line: untranslated 3' region, grey arrow: 500 nucleotide jump, black arrow: 6000 nucleotide jump.



Supplemental Figure 3. Selenoprotein genes expression in zebrafish brain with no difference during the different life stages. Gene transcription was quantified by number of read nucleotide matches with each selenoprotein gene CDS. Data were normalized by the target gene size and RNA-Seq experiment size. Transcription levels are expressed in relative fold changes to an average of *uba52* and *rlp13a* transcription. Results are expressed as average of 10 replicates ± mean standard error and analyzed by one-way ANOVA followed by Tukey multiple comparison test.

5 CONCLUSÃO E PERSPECTIVAS

- O conjunto de dados deste trabalho é a mais atual revisão deste tipo de gene em peixe-zebra a nível de transcrito.
- Entre os tecidos, o cérebro apresentou maior diversidade na transcrição de selenoproteínas.
- A pesquisa demonstrou pela primeira vez uma tendência a altos níveis de transcrição de selenoproteínas em cérebro de peixe-zebra adulto.
- Entre os genes para selenoproteínas, destacam-se *dio2, gpx1a, gpx4a, gpx4b, msrb1b, selenof, selenop, selenot1a, selenot2 selenow1* e *selenow2a* como bons genes alvos para estudos translacionais de selenoproteínas.
- Ainda é necessário determinar o efeito da distribuição sexual sobre a transcrição de genes que codificam selenoproteínas, assim como a diferença entre cepas.
- Trabalhos futuros devem explorar a ausência de elemento SECIS na sequência anotada para *selenoj*.
- A ativação de genes em outros estágios de vida e tecidos poderá elucidar mais alvos e indicar funções de genes pouco presentes nos transcriptomas estudados neste trabalho.

6 REFERÊNCIAS BIBLIOGRÁFICAS

- AMSTERDAM, A.; NISSEN, R. M.; SUN, Z. et al. Identification of 315 genes essential for early zebrafish development. **Proc Natl Acad Sci** 101:12792-12797. 2004.
- BARBAZUK, W. B.; KORF, I.; KADAVI, C. et al. The syntenic relationship of the zebrafish and human genomes. **Genome Res** 1351–1358. 2000.
- BENNER, M. J.; DREW, R. E.; HARDY, R. W., et al. Robison BD. Zebrafish (*Danio rerio*) vary by strain and sex in their behavioral and transcriptional responses to selenium supplementation. **Comp Biochem Physiol A Mol Integr Physiol** 157:310-8. 2010.
- BENNER, M. J.; SETTLES, M. L.; MURDOCH, G. K. et al. Sex-specific transcriptional responses of the zebrafish (*Danio rerio*) brain selenoproteome to acute sodium selenite supplementation. **Physiol Genomics** 45:653-66. 2013.
- BETANCOR, M. B.; ALMAIDA-PAGÁN, P. F.; SPRAGUE M. et al. Roles of selenoprotein antioxidant protection in zebrafish, *Danio rerio*, subjected to dietary oxidative stress. **Fish Physiol Biochem** 41:705-20. 2015.
- BIANCO, A. C., LARSEN, P. R. Selenium, deiodinases and endocrine function. In: Hatfield DL, Berry MJ, Gladyshev VN, editors. Selenium: Its molecular biology and role in human health. **Springer**. pp. 207–219. 2006.
- BIFANO, A.L., T. ATASSI, T. FERRARA and D. M. Driscoll, Identification of nucleotides and amino acids that mediate the interaction between ribosomal protein L30 and the SECIS element. **BMC Mol. Biol**., 2013, 14, 12
- BÖSL, M.R.; TAKAKU, K.; OSHIMA, M.; Nishimura, S. Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp). **Proc. Natl. Acad. Sci**. 94:5532–5534. 1997.
- BUDIMAN, M. E.; BUBENIK, J. L.; MINIARD, A. C. et al. Eukaryotic Initiation Factor 4a3 Is a Selenium-Regulated RNA-Binding Protein that Selectively Inhibits Selenocysteine Incorporation. **Mol. Cell** 35:479–489. 2009.
- BUETTNER, C., HARNEY, J. W., BERRY, M. J. The *Caenorhabditis elegans* Homologue of Thioredoxin Reductase Contains a Selenocysteine Insertion Sequence (SECIS) Element That Differs from Mammalian SECIS Elements but Directs Selenocysteine Incorporation. **Journal of Biological Chemistry**, 274:21598–21602. 1999.

- BURK, R. F., & HILL, K. E. Selenoprotein P-expression, functions, and roles in mammals. **Biochimica et biophysica acta**, 1790(11), 1441–1447. 2009
- BURK, R. F.; HILL, K. E. Regulation of Selenium Metabolism and Transport. **Annu. Rev. Nutr**.35:109-134. 2015.
- CAO, J., NAVIS, A., COX, B. D., et al. Single epicardial cell transcriptome sequencing identifies Caveolin-1 as an essential factor in zebrafish heart regeneration. **Development** 143(2):232-43. 2016.
- CARDOSO, B. R.; ROBERTS, B. R.; BUSH, A. I. et al. Selenium, selenoproteins and neurodegenerative diseases. **Metallomics** 7:1213-1228. 2015.
- CASTELLANO, S.; ANDRES, A. M.; BOSCH, E., et al. Low exchangeability of selenocysteine, the 21st amino acid, in vertebrate proteins. **Mol Biol Evol** 26:2031-2040. 2009.
- CASTELLANO, S.; LOBANOV, A.V.; Chapple, C. et al. Diversity and functional plasticity of eukaryotic selenoproteins: Identification and characterization of the SelJ family. **Proc Natl Acad Sci** 102(45):16188-16193. 2005.
- CASTELLANO, S.; MOROZOVA, N.; MOREY, M. et al. In silico identification of novel selenoproteins in the Drosophila melanogaster genome. **EMBO Rep** 2:697-702. 2001
- CHAPPLE, C. E.; GUIGO, R. Relaxation of selective constraints causes independent selenoprotein extinction in insect genomes. **PLoS One** 3:e2968. 2008.
- CONE, J. E., DEL RÍO, R. M., DAVIS, J. N., et al. Chemical characterization of the selenoprotein component of clostridial glycine reductase: identification of selenocysteine as the organoselenium moiety. **Proc. Natl. Acad. Sci**. U. S. A. 73, 2659−2663. 1976.
- COX, A.G., TSOMIDES, A., KIM, A.J., et al. Selenoprotein H is an essential regulator of redox homeostasis that cooperates with p53 in development and tumorigenesis. **Proc. Natl. Acad. Sci**. USA. 113:5562-71. 2016.
- Crooks G. E.; Hon G.; Chandonia J. M. et al. WebLogo: A sequence logo generator, **Genome Research.** 14:1188-1190. 2004.
- Dikiy A.; Novoselov S. V.; Fomenko, D.E. et al. SelT, SelW, SelH, and Rdx12: genomics and molecular insights into the functions of selenoproteins of a novel thioredoxin-like family. **Biochemistry** 46:6871–6882. 2007.

- DOLGOVA, N.V.; NEHZATI, S.; MACDONALD, T. C. et al. Disruption of selenium transport and function is a major contributor to mercury toxicity in zebrafish larvae. **Metallomics**. 11:621-631. 2019.
- DREW, R.E.; SETTLES, M. L.; CHURCHILL, E. J. et al. Brain transcriptome variation among behaviorally distinct strains of zebrafish (*Danio rerio*). **BMC Genomics**. 13:323. 2012.
- DUDKIEWICZ, M.; SZCZEPIŃSKA, T.; GRYNBERG, M. et al. A Novel Protein Kinase-Like Domain in a Selenoprotein, Widespread in the Tree of Life. **PLoS ONE** 7(2): e32138. 2012.
- DUMONT, E.; VANHAECKE, F.; CORNELIS, R. Selenium speciation from food source to metabolites: a critical review. **Anal Bioanal Chem**. 385:1304–23. 2006
- ERICKSON, T.; NICOLSON, T. Identification of sensory hair-cell transcripts by thiouracil-tagging in zebrafish. **BMC Genomics**. 16:842. 2015.
- FAGEGALTIER D.; LESCURE A.; WALCZAK P. et al. Structural analysis of new local features in SECIS RNA hairpins. **Nucleic Acids Res**.28(14):2679-89. 2000
- FERGUSON, A., LABUNSKYY, V. M., FOMENKO, D. E. et al. NMR Structures of the Selenoproteins Sep15 and SelM Reveal Redox Activity of a New Thioredoxin-like Family. **The Journal of Biological Chemistry** 281, 3536-3543. 2006.
- FLOHE, L.; GÜNZLER, W.; SCHOCK, H. Glutathione peroxidase: A selenoenzyme **FEBS Lett**., 32, 132–134 1973
- FREDERICKS, G. J.; HOFFMANN, P. R. Selenoprotein K and protein palmitoylation. **Antioxid Redox Signal**. 23(10):854-62. 2015.
- GLADYSHEV, V. N.; ARNÉR, E. S., BERRY, M. J., et al. Selenoprotein Gene Nomenclature. **The Journal of biological chemistry**, 291(46), 24036–24040. 2016 .
- GOBLER, C. J.; LOBANOV, A.V.; TANG, Y.Z. et al. The central role of selenium in the biochemistry and ecology of the harmful pelagophyte, *Aureococcus anophagefferens*. **ISME J** 7: 1333–1343, 2013.
- GOLDSMITH, P. Zebrafish as a pharmacological tool: The how, why and when. **Curr Opin Pharmacol**, p. 504–512, 2004.

- GRIM, J. M.; HYNDMAN, K.A.; KRISKA, T. et al. Relationship between oxidizable fatty acid content and level of antioxidant glutathione peroxidases in marine fish. **The Journal of Experimental Biology**. 214 (22): 3751–3759. 2011.
- GRUNDNER-CULEMANN, E.; MARTIN, G. W.,3rd; HARNEY, J. W. et al. Two distinct SECIS structures capable of directing selenocysteine incorporation in eukaryotes. **RNA** 5(5), 625–635. 2019.
- GUO, C., CHEN, X., SONG, H., et al. Intrinsic Expression of a Multiexon Type 3 Deiodinase Gene Controls Zebrafish Embryo Size. **Endocrinology**. 155(10):4069-80. 2014.
- GUO, L., YANG, W., HUANG, Q., et al. Selenocysteine-Specific Mass Spectrometry Reveals Tissue-Distinct Selenoproteomes and Candidate Selenoproteins. **Cell Chemical Biology**. 2018
- HAMILTON, S. J. Review of selenium toxicity in the aquatic food chain**. Sci Total Environ** 326:1–31. 2004.
- HAN, S. et al. "Characterization of mammalian selenoprotein o: a redox-active mitochondrial protein." **PloS one** vol. 9,4 e95518. 2014.
- HARTIKAINEN, H.L. Biochemistry of selenium and its impact on food chain quality and human health. **J. Trace Elem. Med. Biol**. 18, 309–318. 2015
- HATFIELD, D.L.; GLADYSHEV, V.N. How selenium has altered our understanding of the genetic code. M**ol. Cell. Biol**. 22, 3565–3576. 2002
- HEBERLE, H.; MEIRELLES, G. V.; DA SILVA, F. R. et al. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. **BMC Bioinformatics** 16:169. 2015.
- HEIDEN, T.C.; STRUBLE, C.A.; RISE, M.L., et al. Molecular targets of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) within the zebrafish ovary: Insights into TCDD-induced endocrine disruption and reproductive toxicity. **Reproductive toxicology** 25:47-57. 2008.
- HEIJLEN, M.; HOUBRECHTS, A. M.; BAGCI E. A. et al. Knockdown of Type 3 Iodothyronine Deiodinase Severely Perturbs Both Embryonic and Early Larval Development in Zebrafish, **Endocrinology**, 155:1547–1559. 2013

- HENNEBERRY, A. L.; MCMASTER, C. R. Cloning and expression of a human choline/ethanolaminephosphotransferase: synthesis of phosphatidylcholine and phosphatidylethanolamine. **Biochem J** 339: 291–298. 1999.
- HOLBEN, D.H.; SMITH, A.M. The diverse role of selenium within selenoproteins: A review. Jo**urnal of the American Dietetic Association** 99:836-843. 1999.
- HOUSECROFT, C.; SHARPE, A.G. Inorganic chemistry. 4th ed. **Harlow: Pearson Education Limited**; chapter 15. 2012;
- HOWE, K.; CLARK, M. D.; TORROJA, C. F. et al. The zebrafish reference genome sequence and its relationship to the human genome. **Nature**, 496(7446), 2013.
- JAPHA, A. Experimental nonnulla de vi selenii in organismum animalen. **Dissertation**, Halle. 1842 (Cit. Moxon & Rhian, 1943.)
- JUKES T. H. Selenium, an "essential poison". J Appl Biochem 5:233-234. 198
- JURYNEC, M.J. et al. “Selenoprotein N is required for ryanodine receptor calcium release channel activity in human and zebrafish muscle.” **Proceedings of the National Academy of Sciences of the United States of America** vol. 105,34:12485-90. 2008
- KATOH, K.; MISAWA, K.; KUMA, K. et al. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. **Nucleic acids research**, 30(14), 3059–3066. 2002.
- KERVESTIN, S.; JACOBSON, A. NMD: A multifaceted response to premature translational termination. **Nat. Rev. Mol. Cell. Biol**. 13, 700–712. 2012
- KESHAN DISEASE RESEARCH GROUP OF THE CHINESE ACADEMY OF MEDICAL SCIENCES. Observations on effect of sodium selenite in prevention of Keshan disease. **Chin. Med. J.** 92, 471−476. 1979
- KIJIMA, Y.; WANTONG, W.; IGARASHI, Y. et al. Age-associated different transcriptome profiling in zebrafish and rat: insight into diversity of vertebrate aging. **bioRxiv** 478438; 2018
- KOIVISTOINEN, P.; HUTTUNEN, J. K. Selenium in food and nutrition in Finland: An overview on research and action. **Ann. Clin. Res**. 18, 13−17. 1986
- KRYUKOV GV, et al. Characterization of mammalian selenoproteomes. **Science**, 2003 May 30. PMID 12775843

- KRYUKOV GV, KRYUKOV VM, GLADYSHEV VN. New mammalian selenocysteine-containing proteins identified with an algorithm that searches for selenocysteine insertion sequence elements. **J Biol Chem** 274:33888-33897. 1999.
- KRYUKOV, G.V. and GLADYSHEV, V.N. Selenium metabolism in zebrafish: multiplicity of selenoprotein genes and expression of a protein containing 17 selenocysteine residues. **Genes to cells : devoted to molecular & cellular mechanisms**. 5(12):1049-1060. 2000.
- LABUNSKYY, V. M.; HATFIELD, D. L.; Gladyshev, V. N. Selenoproteins: molecular pathways and physiological roles. **Physiol. Rev**., 94:739–777. 2014.
- LABUNSKYY, V. M.; YOO, M.; HATFIELD, D. L., et al. Sep15, a Thioredoxin-like Selenoprotein, Is Involved in the Unfolded Protein Response and Differentially Regulated by Adaptive and Acute ER Stresses. **Biochemistry** 48:8458-8465. 2009.
- LATRECHE, L.; JEAN-JEAN, O.; DRISCOLL, D.M.; Chavatte, L. Novel structural determinants in human SECIS elements modulate the translational recoding of UGA as selenocysteine. **Nucleic Acids Res**. 37, 5868–5880. 2009
- LEE, J. H.; PARK, K. J.; JANG, J. K. et al. Selenoprotein S-dependent Selenoprotein K Binding to p97(VCP) Protein Is Essential for Endoplasmic Reticulum-associated Degradation. **The Journal of biological chemistry**, 290(50), 29941–29952. doi:10.1074/jbc.M115.680215. 2015.
- LEMLY, A.D. Assessing the toxic threat of selenium to fish and aquatic birds. **Environ Monit Assess**.43:19–35 1996.
- LESCURE, A.; GAUTHERET, D.; CARBON, P. et al. Novel selenoproteins identified in silico and in vivo by using a conserved RNA structural motif. **J Biol Chem** 274:38147-38154. 1999.
- LIESCHKE, G.J.; CURRIE P.D. Animal models of human disease: zebrafish swim into view. **Nat Rev Genet**, v. 8, p. 353–367, 2007.
- LOBANOV, A. V.; HATFIELD, D. L.; GLADYSHEV, V. N. Selenoproteinless animals: selenophosphate synthetase SPS1 functions in a pathway unrelated to selenocysteine biosynthesis. **Protein Sci**. 17,129. 176−182. 2008

- LOBANOV, A.V.; HATFIELD, D.L.; GLADYSHEV, V.N. 2008. Reduced reliance on the trace element selenium during evolution of mammals. **Genome Biol** 9:R62
- LOW, S.L.; GRUNDNER-CULEMANN, E.; HARNEY, J.W.; Berry, M.J. SECIS-SBP2 interactions dictate selenocysteine incorporation efficiency and selenoprotein hierarchy. **EMBO J**. 2000, 19, 6882–6890
- MARELLI, F.; CARRA, S.; AGOSTINI, M. et al. Patterns of thyroid hormone receptor expression in zebrafish and generation of a novel model of resistance to thyroid hormone action. **Molecular and Cellular Endocrinology**. 424:102-17. 2016.
- MARIOTTI, M. A.; LOBANOV, V.; GUIGO, R. et al. SECISearch3 and Seblastian: new tools for prediction of SECIS elements and selenoproteins. **Nucl. Acids Res**. 2013.
- MARIOTTI, M.; RIDGE, P. G.; ZHANG, Y. et al. Composition and Evolution of the Vertebrate and Mammalian Selenoproteomes. **PLoS ONE** 7(3):e33066. 2012.
- MARTIN, G. W., 3rd; HARNEY, J. W.; BERRY, M. J. Selenecysteine incorporation in eukaryotes: Insights into mechanism and efficiency from sequence, structure, and spacing proximity studies of the type I deiodinase SECIS element. **RNA** 2, 171–182. 1996.
- MARTIN-ROMERO, F. J.; KRYUKOV, G. V.; LOBANOV, A. V. et al. Selenium metabolism in *Drosophila*: selenoproteins, selenoprotein mRNA expression, fertility, and mortality. **J Biol Chem** 276:29798-29804 2001.
- MENDELEV, N. et al. "Upregulation of human selenoprotein H in murine hippocampal neuronal cells promotes mitochondrial biogenesis and functional performance." **Mitochondrion** 11.1 (2011): 76-82.
- MENDIETA-SERRANO, M. A.; SCHNABEL-PERAZA, D.; LOMELÍ, H. et al. Spatial and temporal expression of zebrafish glutathione peroxidase 4 a and b genes during early embryo development. **Gene Expression Patterns**. 19(1-2):98-107. 2015.
- MOGHADASZADEH, B.; BEGGS, A. H. "Selenoproteins and their impact on human health through diverse physiological pathways." **Physiology (Bethesda, Md.)** vol. 21 (2006): 307-15.
- MORENO, M., BERRY, M.J.; HORST, C. et al. Activation and inactivation of thyroid hormone by type I iodothyronine deiodinase.**FEBS Lett**. 1994 May 16; 344(2-3):143-6.

- MORTAZAVI, A.; WILLIAMS, B. A.; MCCUE, K. et al. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature methods. 2008 Jul 1;5(7):621-8.
- MOXON A.L.; RHIAN, M. 1943. Selenium poisoning. **Physiol. Reviews** 23(4):305-337. 1943.
- NOH, O. J., PARK, Y. H., CHUNG, Y. W., et al. Transcriptional regulation of selenoprotein W by MyoD during early skeletal muscle differentiation. **Journal of Biological Chemistry**, 285(52), 40496-40507. 2010.
- NOVOSELOV, S. V. et al. Identification and characterization of Fep15, a new selenocysteine-containing member of the Sep15 protein family. **The Biochemical journal** 394:575-579. 2006.
- OLDFIELD, J. E. The selenium story: some reflections on the "moon-metal". **N. Z. Vet. J.** 22:85−94. 1974.
- OLIVEIRA, C. S.; PICCOLI, B. C.; ASCHNER, M. et al. Chemical Speciation of Selenium and Mercury as Determinant of Their Neurotoxicity. **Neurotoxicity of Metals**, 53–83. 2017.
- PAINTER, E. P.; FRANKE, K. W. SELENIUM IN PROTEINS FROM TOXIC FOODSTUFFS: III. THE REMOVAL OF SELENIUM FROM TOXIC PROTEIN HIDROLYSATES J. Biol. Chem. 111:643-651. 1935.
- PAPP, L. V.; LU, J.; HOLMGREN, A., et al. From Selenium to Selenoproteins: Synthesis, Identity, and Their Role in Human Health. **Antioxidants & Redox Signaling**, 9:775–806. 2007.
- PENGLASE, S.; HAMRE, K.; ELLINGSEN S. Selenium prevents downregulation of antioxidant selenoprotein genes by methylmercury. **Free Radic Biol Med**. 75:95-104. 2014a
- PENGLASE, S.; HAMRE, K.; RASINGER, J. D; et al. Selenium status affects selenoprotein expression, reproduction, and F_1 generation locomotor activity in zebrafish (*Danio rerio*). **Br J Nutr** 111:1918-31. 2014b.
- PINSENT, J. The need for selenite and molybdate in the formation of formate dehydrogenases by members of the *Coliaerogenes* group of bacteria. **Biochem J** 57:10−16. 1954.

- RAJPUT, B.; PRUITT, K. D.; MURPHY, T. D. RefSeq curation and annotation of stop codon recoding in vertebrates. **Nucleic acids research**, 47:594–606. 2019.
- REEVES, M.A.; BELLINGER, F. A.; BERRY, M. J. Antioxidants & Redox Signaling.Apr 2010.**ahead of print** http://doi.org/10.1089/ars.2009.2883
- REICH, H. J.; HONDAL, R. J. Why Nature Chose Selenium. **ACS Chemical Biology**, 11:821–841. 2016.
- ROBINSON, D. N.; COOLEY, L. Examination of the function of two kelch proteins generated by stop codon suppression. **Development** 124:1405-1417. 1997.
- ROBINSON, W. O. **J. Assn. Of. Agric. Chem**., 16, 423 1933. (Cit: Painter & Franke, 1935)
- ROCHA, J. B. T.; PICCOLI, B. C.; OLIVEIRA, C.S. Biological and chemical interest in selenium: a brief historical account. **ARKIVOC** 5550190.p009.784. 2017
- ROMAGNÉ, F.; SANTESMASSES, D.; WHITE, L. et al. SelenoDB 2.0: annotation of selenoprotein genes in animals and their genetic diversity in humans. **Nucleic Acids Res**, 42:437-443. 2014.
- ROSENFELD, I.; BEATH, O.A. Selenium Geobotany, Biochemistry, Toxicity and Nutrition. Economic Botany, 20, 340-343. 1964
- SCHWARZ, K.; FOLTZ, C. M. Selenium as an integral part of factor 3 against dietary liver degeneration. **J Am Chem Soc** 79:3292−3293. 1957
- SELINUS, O.; ALLOWAY, B.; CENTENO, J. A. et al. Essentials of Medical Geology: Impacts of the Natural Environment on Public Health. **Burlington**: Elsevier Academic Press. 2005.
- SENGUPTA, A.; CARLSON, B. A.; LABUNSKYY, V. M. et al. Selenoprotein T deficiency alters cell adhesion and elevates selenoprotein W expression in murine fibroblast cells. **Biochemistry and Cell Biology**, 87, 953-961. 2009.
- SHAMBERGER, R. J. Selenium and health. In Trace elements in health: A review of current issues. **Rose, J., Ed.** 167−181, 1983
- SHCHEDRINA, V. A.; EVERLEY, R. A.; ZHANG, Y. et al. (2011). Selenoprotein K binds multiprotein complexes and is involved in the regulation of endoplasmic reticulum homeostasis. **The Journal of biological chemistry** 286:42937–42948. 2011.

- SHIN, J. T.; FISHMAN, M. C. From Zebrafish to human: modular medical models. **Annu Rev Genomics Hum Genet** 3:311–340. 2002.
- SUNDE, R.A.; RAINES, A.M.; BARNES, K.M.; et al. Selenium status highly regulates selenoprotein mRNA levels for only a subset of the selenoproteins in the selenoproteome. **Biosci Rep** 29:329–338. 2009.
- TAN, J. A.; HOU, S. F.; ZHU, W. et al. The Keshan disease in China: a study of the geographical epidemiology. **Acta Geographica Sinica** 34:85−104. 1979.
- TAYLOR, J. S.; BRAASCH, I.; FRICKEY, T. et al. Genome duplication, a trait shared by 22000 species of ray-finned fish. **Genome research** 13:382–390. 2003.
- TROFAST, J. Berzelius' Discovery of Selenium. **Chemistry International** 33:16−19. 2011.
- VIHTELIC, T. S.; FADOOL, J. M.; GAO, J.; et al. Expressed sequence tag analysis of zebrafish eye tissues for NEIBank. **Molecular Vision**. 11:1083-1100. 2005.
- WU, R.; SHEN, Q.; NEWBURGER, P.E. Recognition and binding of the human selenocysteine insertion sequence by nucleolin. **J. Cell. Biochem**. 77:507–516. 2000.
- YAMASHITA, Y.; YABU, T.; TOUHATA, K. et al. Purification and characterization of glutathione peroxidase 1 in the red muscle of Pacific bluefin tuna *Thunnus orientalis*. **Fisheries science** 78:407-413. 2012.
- YAN, J.; FEI, Y.; HAN, Y. et al. Selenoprotein O deficiencies suppress chondrogenic differentiation of ATDC5 cells. **Cell Biol Int**, 40:1033-1040. 2016.
- ZHANG, L.; ZHU, J. H.; ZHANG X.; Cheng WH. The Thioredoxin-Like Family of Selenoproteins: Implications in Aging and Age-Related Degeneration. **Biol Trace Elem Res**. 188189-195. 2018.
- ZHENG, W.; XU, H.; LAM, S.H. et al. Transcriptomic analyses of sexual dimorphism of the zebrafish liver and the effect of sex hormones. **PLOS ONE**. 8 (1): e53562. 2013.