UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOTECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOQUÍMICA

Estudo dos efeitos fisiopatológicos e moleculares do *knockdown* do gene CG15105 em cérebro de *Drosophila melanogaster*

Discente: Matheus Henrique Silva

Orientador: Prof. Dr. Carlos Ueira-Vieira

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2022, Uberlândia-MG

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ATA DE DEFESA - PÓS-GRADUAÇÃO

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Data:	Trinta e um de janeiro de dois mil e vinte e dois.	Hora de início:	09:20h	Hora de encerramento:	09:50h
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Projeto de Pesquisa de vinculação:	RNASEQ de cérebro de modelos biológicos de doença de *Alzheimer* e sua utilizaçao na validação de Peptídeo neuromoduladores.				

Aos trinta e um dias do mês de janeiro de dois mil e vinte e dois, às 09:20 horas, reuniu-se via web conferência pela plataforma *Cisco Webex*, em conformidade com a Portaria nº 36, de 19 de março de 2020 da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES e Resolução de nº 06/2020 do Conselho de Pesquisa e Pós-graduação pela Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Genética e Bioquímica, assim composta: Professores Doutores: Carlos Ueira Vieira (Orientador), Anderson de Oliveira Souza e Renata Graciele Zanon. A participação dos dois últimos se deu por epístola. Iniciando os trabalhos o (a) presidente Dr (a). Carlos Ueira Vieira apresentou a Comissão Examinadora e o candidato(a), agradeceu a presença dos participantes, e concedeu ao Discente a palavra para a exposição do seu trabalho. A duração da apresentação do Discente e o tempo de arguição e resposta foram conforme as normas do Programa. A seguir o senhor(a) presidente procedeu a leitura das epístolas enviadas pelos membros da banca. Em seguida os membros presentes, passaram a arguir o(a) candidato(a). Ultimada a leitura das epístolas e a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

(A) PROVADO.

Esta defesa de Dissertação de Mestrado é parte dos requisitos necessários à obtenção do título de Mestre. O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU. Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

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Dedicatória

Dedico este trabalho a memória de meus avós Sebastião e Geralda.

Epígrafe

"Juro não faço por mal
Logo você que diz me conhecer tão bem
Devia saber que isso é tão normal
Ando sem tempo pra você, mas amanhã eu passo pra te ver
Sei que 'ta' difícil acreditar em mim
Até os meus amigos dizem que eu sumi
Assumo essa é a vida que eu escolhi
A gente não 'ta' perto mas 'to' longe de esquecer"

(Lagum)

LAGUM. Oi. Sony Music Entertainment. Belo Horizonte. Disponível em: https://www.youtube.com/watch?v=JsbvyRXpNmE Acesso em 28 de dezembro de 2021

SUMÁRIO

Apresentação

O presente trabalho tem como enfoque estudar aspectos genéticos e bioquímicos relacionados a fisiopatologia da neurodegeneração, utilizando estudos *in silico* e *in vivo* (*Drosophila melanogaster*).

O capítulo 1 se trata da fundamentação teórica. Nele constam informações retiradas de acervos de literatura científica a respeito de conhecimentos necessários para a compreensão do presente trabalho. Este capítulo contém informações sobre doenças neurodegenerativas, o modelo animal utilizado (a *D. melanogaster*) e pôr fim a técnica de interferência de RNA.

Já o capítulo 2 é o artigo científico referente a pesquisa realizada. Neste capítulo constam estudos experimentais realizados com a finalidade de compreender o papel do gene CG15105 nos processos fisiopatológicos da neurodegeneração, e também, as interações moleculares e contribuições da mesma para processos relacionados a neurodegeneração.

RESUMO

A neurodegeneração é um processo fisiopatológico leva ao comprometimento do Sistema Nervoso, é comumente relacionado com o envelhecimento por se tratar de danos cumulativos ao longo do tempo de vida. Com o aumento da expectativa de vida da população mundial o estudo da neurodegeneração se tornou uma prioridade mundial. A doença de Alzheimer é uma das mais comuns dentre as doenças neurodegenerativas, um dos eventos fisiopatológicos dessa doença é acúmulo do peptídeo β-amiloide, devido ao desequilíbrio entre produção e degradação do mesmo. O acúmulo de β-amiloide se relaciona com outras alterações como a redução da expressão do gene CG15105 (tn), que codifica uma proteína envolvida em processos de degradação proteica por meio da ubiquitinação. Devido a isso, o presente trabalho teve como objetivo explorar os efeitos do gene CG15105 em cérebro de *Drosophila melanogaster*. Por meio do uso da técnica de *knockdown* genético com iRNA em cérebro de moscas e análises histopatológicas e comportamentais foi observado que o *knockdown* do gene CG15105 reduz a atividade locomotora, e o *knockdown* causa degeneração da morfologia cerebral. E os achados dos estudos *in silico* do gene CG15105, mostraram que sua atividade está relacionada principalmente com a ubiquinação. E alguns dos genes da rede de interação do CG15105 tem atividade no citoesqueleto e apresentam alteração transcricionais em cérebro de moscas relacionadas aos processos de envelhecimento e presença do peptídeo β-amiloide.

Palavras-chave: Neurodegeneração, *Drosophila melanogaster*, iRNA, *tn* e ubiquitinação.

ABSTRACT

Neurodegeneration is a pathophysiological process leading to an impaired nervous system, it is caused by cumulative damage over a lifetime and it is commonly related to aging. With the increase in life expectancy of the world population, the study of neurodegeneration has become a world priority. Alzheimer's disease is one of the most common neurodegenerative diseases. A pathophysiological event of this disease is the accumulation of the β-amyloid peptide, caused by the imbalance between production and degradation. The accumulation of β-amyloid is related to other alterations such as the expression reduction of gene CG15105 (tn), which encode proteins involved in protein degradation processes through ubiquitination. In this way, the present work aimed to explore the effects of the CG15105 gene on the brain of Drosophila melanogaster.

Through the use of the technique of genetic knockdown with iRNA in the brain of flies and histopathological and behavioral analyses it was observed that the knockdown of CG15105 gene reduce locomotor activity, and the knockdown of CG15105 causes morphological degeneration of brain. And the findings of the *in silico* studies of the CG15105 show that its activity is mainly related to ubiquitination. And some of the genes in the CG15105 interaction network that have cytoskeleton functions present transcriptional alterations in the brain of flies related to aging processes and the presence of the β-amyloid peptide.

Key-words: Neurodegeneration, *Drosophila melanogaster*, iRNA, *tn*, and ubiquitination.

CAPÍTULO 1: Fundamentação teórica

Doenças neurodegenerativas

Nas doenças neurodegenerativas ocorre o comprometimento de funções do Sistema Nervoso (SN) devido a lesões progressivas nas células que compõem este sistema. Essas lesões podem ter como etiologia o acúmulo proteico em células nervosas, levando ao comprometimento funcional e estrutural do SN. Além disso essas doenças podem compartilhar processos fisiopatológicos semelhantes na neuroinflamação, disfunções na ubiquitinação e estresse proteotóxico (DUGGER; DICKSON, 2017).

As doenças neurodegenerativas são classificadas de acordo com as alterações moleculares, anatômicas e manifestações clinicas. Alguns exemplos são: a Doença Frontotemporal, causada por alterações anatômicas nos lóbulos temporais e frontal; a Doença de Parkinson, caracterizada pela desordem motora e presença de corpos de Lewy; e, por fim, a doença de Alzheimer, que apresenta acúmulo proteico de beta-amiloide e proteína Tau e perda de memória (KOVACS, 2018).

Essas doenças podem causar perda progressiva da capacidade cognitiva caracterizando uma demência. Pessoas com demência tem problemas de memória, que dificultam o reconhecimento pessoas e lugares familiares, também apresentam dificuldades de comunicação, aprendizado, controle emocional, alterações de humor e problemas com atividades de autocuidado (WORLD HEALTH ORGANIZATION, 2021).

Em 2017 a Organização Mundial de Saúde reconheceu a demência como uma prioridade da saúde pública mundial, e propôs que os governos fomentassem e realizassem pesquisas para desenvolvimento de tecnologias direcionadas ao diagnóstico e tratamento dos pacientes com demência (WORLD HEALTH ORGANIZATION, 2017).

Dentre as causas de demência a mais comum é a Doença de Alzheimer (DA) sendo responsável por 60 a 70% dos casos (ERKKINEN; KIM; GESCHWIND, 2018; WORLD HEALTH ORGANIZATION, 2021). Foi estimado que cerca de 6,1 milhões de pessoas eram portadoras da doença de Alzheimer no Estados Unidos da América(EUA) em 2011 com prevalência de 14,7% (RAJAN et al., 2019). O censo dos EUA estimou que em 2020 6,07 milhões de pessoas viviam com DA clínica e a perspectiva é que em 2060 este

número seja de 13,85 milhões de portadores da Doença de Alzheimer clínica (RAJAN et al., 2021). Quando analisado os índices da DA em escala mundial Brasil é o segundo pais com maior prevalência da doença (1037 por 100.000) (NICHOLS et al., 2019).

O principal sintoma da DA é a amnesia, que se manifesta com esquecimentos de memória recente e evolui para perdas mais severas. Outras manifestações clínicas também podem ser observadas, dentre elas: problemas cognitivos relacionados a criatividade, ansiedade, alterações sensoriais e alterações de humor (ATRI, 2019).

A Sociedade Americana de Psiquiatria definiu que os critérios para o diagnóstico da doença de Alzheimer são através da sintomatologia e do histórico familiar do paciente. Alguns biomarcadores também podem ser utilizados, como a presença de atrofia cortical e presença da placa senil, detectada em exames histopatológicos pós-morte, ou através de exames de tomografia cerebral por emissão de pósitrons (PET), e também, os níveis da peptídeo β-amiloide 42 ou da Tau fosfato no liquido cérebro-espinhal (LCS). Marcados genéticos, também, podem ser usados para auxiliar no diagnóstico como os genes da proteína precursora de amiloide (APP) e presenilina 1 ou 2 (PSEN1 ou PSEN2). Entretanto, esses marcadores biológicos não estão validados ainda, o que acarreta em diagnósticos com certo grau de incerteza, por isso há possibilidade do diagnóstico ser "provável" ou "possível" doença de Alzheimer (AMERICAN PSYCHIATRIC ASSOCIATION, 2014)

A principal característica da fisiopatologia da doença é formação da placa senil, que consiste em aglomeração do peptídeo β-amiloide (βa) e emaranhados neurofibrilares de Tau. A formação do βa ocorre quando a proteína precursora de amiloide (PPA) é clivada pelas enzimas β-secretase e γ-secretase, essa via de clivagem é uma via alternativa e é chamada de via amiloidogênica, comumente esta clivagem ocorre pelas enzimas α-secretase e γ-secretase e leva a formação de peptídeos solúveis que são degradados. O peptídeo βa possui entre 36 e 42 aminoácidos, e tem funções fisiológicas na sinapse e sobrevivência de neurônios, entretanto o desbalanço entre produção e clearance é tóxico e dá origem a formação das placas de βa, o peptídeo βa de 42 aminoácidos é a isoforma mais citotóxica e com maior potencial patogênico. Esse evento ocorre especificamente nos astrócitos, levando a morte destas células e causando a desmeliniezação dos neurônios (WANG et al., 2017).

A via amiloidogênica interfere na fosforilação da proteína Tau. Quando há a formação de βa ocorre a hiperfosforilação da Tau, que se liga as estruturas do citoesqueleto celular e constituem os emaranhados neurofibrilares (KUMAR; ABBAS; ASTER, 2015).

A fisiopatologia da doença está relacionada, ainda, com a desregulação do metabolismo da glicose. O cérebro possui uma alta demanda de glicose em relação aos demais órgãos do corpo, e tal metabolismo corrobora para a formação de memórias, uma vez que os astrócitos convertem glicogênio em lactato e transportam para os neurônios durante o processo de consolidação de memórias de longo prazo (ABOLHASSANI et al., 2017).

Além disso, alguns aspectos genéticos associados a doença de Alzheimer, como os genes da proteína precursora de amiloide, presenilina 1 e 2 que, estão relacionados processos fisiopatológicos que levam ao desenvolvimento do Alzheimer precoce, ou familiar, que se dá pela herança genética de mutações nestes três genes (LANE; HARDY; SCHOTT, 2018). Outro associado a etiologia da doença é o gene da APOE. Esse gene possui três variantes alélicas, ε2, ε3 e ε4. A presença do alelo ε4 está associada a fatores de risco para o desenvolvimento e progressão da doença, enquanto que o alelo ε2 em homozigose está associado a um baixo risco de desenvolvimento da DA (SERRANO-POZO; DAS; HYMAN, 2021).

Um trabalho de nosso grupo de pesquisa, que visava avaliar as alterações transcricionais durante a via amiloidogênica, observou que o CG15105 tem sua transcrição reduzida com o envelhecimento e com a presença do acúmulo de βa. Desta forma ambos os genes podem estar relacionados com processos de degeneração do Sistema Nervos Central, seja esse processo ligado ao envelhecimento ou com a Doença de Alzheimer (DA COSTA SILVA et al., 2022; SILVA, 2019). Este trabalho utilizou como animal modelo a *Drosophila melanogaster*, que é um modelo muito utilizado na área da genética.

Modelo animal: *Drosophila melanogaster*

Em 1910, Morgan publicou um estudo que descreveu uma mutação que causava alteração fenotípica da cor dos olhos da *D. melanogaster*. Neste estudo, Morgan observou a ocorrência de mutações espontâneas que deixavam as moscas com os olhos da cor branca, originalmente da cor vermelha. E ao avaliar a herdabilidade de tal fenótipo ele

chegou à conclusão: a mutação ocorria no cromossomo X e era recessiva (STEPHENSON; METCALFE, 2013).

O estudo de Morgan foi um precursor de vários trabalhos que revelaram mecanismos genéticos até então desconhecidos. E dessa forma o uso da *D. melanogaster* como modelo animal de estudo foi ampliado possibilitando encontrar grupos de pesquisa, bancos de dados e bancos de linhagens com diferentes linhagens da mosca como o Estoque Central de Bloomington (banco de linhagens de *Drosophilas*) e o Flybase (banco de dados científicos com informações desde ciclo de vida e alimentação até informações genéticas complexas sobre a mosca) ("Bloomington Drosophila Stock Center", [s.d.]; LARKIN et al., 2021).

A *D. melanogaster* é um modelo animal amplamente utilizado na genética devido ao ciclo de vida curto e por possuir um genoma pequeno em relação a outros animais como humanos e murinos. A *D. melanogaster* possui quatro pares de cromossomos sendo o primeiro o par de cromossomos sexuais e os outros três, os autossômicos. O dimorfismo sexual ocorre devido a quantidade de cromossomos X que a mosca possui, sendo dois (XX) para fêmeas e um (XY) para machos (YAMAGUCHI; YOSHIDA, 2018).

Os primeiros estudos utilizando este modelo revelaram mecanismo genéticos importantes em eucariotos. Com a otimização do uso do modelo foram criadas moscas humanizadas com a finalidade de mimetizar as condições de doenças humanas, permitindo a aplicação das moscas como modelo de estudo. Além de apresentar um ciclo de vida curto, levando cerca de 9 a 10 dias para o desenvolvimento do estágio de ovo até o adulto (FLATT, 2020).

Uma das metodologias de construção de moscas transgênicas é o P-elemento. Ele é um elemento de transposição do DNA que ocorre naturalmente na mosca e em suas extremidades 3' e 5' possui sequências repetitivas que vão se ligar ao DNA genômico com o auxílio de uma transposase que cliva o DNA onde é possível a ligação das sequências repetitivas. Para ser utilizada como ferramenta de edição gênica basta incluir a sequência heteróloga entre as sequências repetitivas, e com o auxílio da transposase e da maquinaria de reparo de DNA a sequência heteróloga será inserida no DNA genômico da mosca (RIO, 1991; WEILGUNY et al., 2020).

Na sequência introduzida é necessário inserir algum marcador fenotípico para avaliar o sucesso da técnica, na maioria das vezes é utilizado a marcação da cor dos olhos.

Para isso são utilizadas moscas de olhos brancos e é introduzido uma sequência que contém o gene para dar a coloração vermelha as moscas. E desta forma é possível separar as moscas que realmente possuem a sequência heteróloga das moscas em que a técnica falhou e não houve introdução da sequência de interesse (KLEMENZ; WEBER; GEHRING, 1987; OSANAI-FUTAHASHI et al., 2012).

Para inserir essa ferramenta de edição gênica é utilizada a técnica de microinjeção em ovos de *D. melanosgater*. Nesta técnica são coletados ovos de moscas e é aplicada uma microinjeção, contendo o elemento P e a transposase, na porção distal dos ovos. Desta forma, as células germinativas das moscas serão afetadas, e quando a mosca eclodir ela carregará a sequência transgênica em suas células germinativas e passará os genes transgênicos para sua prole. Portanto, as moscas transgênicas que serão utilizadas são a prole da mosca que recebeu a microinjeção (LIM et al., 2018; RINGROSE, 2009).

Existem algumas maquinarias que permitem a regulação da expressão heteróloga de genes aplicáveis em *D. melanogaster* transgênicas, dentre elas o sistema UAS-GAL4. Esse sistema é natural de fungos e tem a função de regulação da transcrição de maneira temporal e espacial, ou seja, ele regula em qual fase da vida haverá a transcrição e em qual (quais) tecido (s) será expresso. A GAL4 é uma enzima ligante a sequência UAS para iniciar a transcrição. O transgênico que carrega a sequência GAL4 é chamada de linhagem *Driver*, pois essa linhagem carrega um enhancer a 3’ da sequência GAL4 que irá controlar quando e onde a enzima GAL4 será produzida. E o transgênico com a sequência UAS é a linhagem *responder*, por carregar a sequência a ser expressa a 5’ da sequência UAS, e é transcrita quando GAL4 se liga a UAS (CAYGILL; BRAND, 2016).

Desta forma, é possível realizar cruzamentos entre linhagens *Driver* e *responder* a fim de obter moscas com expressão de genes em momentos específicos do ciclo de vida e em determinados tecidos, permitindo a análise do efeito de genes de acordo com o tecido e fase do desenvolvimento de sua expressão.

Também é possível avaliar o efeito da não expressão de genes por meio do *knockdown* utilizando a técnica de interferência de RNA. Essa técnica consistem em inserir sequências de DNA codificadoras de dsRNA, que irão se ligar a RNA e levar a sua degradação (MOHR, 2014).

Interferência de RNA

A interferência de RNA é um mecanismo de silenciamento genético pós-transcricional, ou seja, um processo impede a tradução de determinado mRNA. Isso ocorre por meio da ação dos dsRNA (double stand RNA), que são moléculas de RNA em fita dupla capazes de circularem no meio extracelular, e quando entram no citoplasma das células são clivados pela enzima Dicer e dando origem aos siRNA. A proteína Argo se liga aos siRNA para formar o complexo de silenciamento por interferência de RNA, este complexo se liga a uma sequência específica do mRNA para que ele seja clivado e, dessa forma, impossibilita a tradução (KANASTY, 2013).

Os dsRNA tem tamanho maior ou igual a 100 pares de base e possuem propriedades que não são observadas em outros tipos de RNAs, como por exemplo a ligação a proteínas específicas e não formação de estruturas tridimensionais de RNA (REICH; BASS, 2019). Em *D. melanogaster*, os dsRNA se ligam a sequências de RNAs virais como objetivo de realizar a degradação do mesmo, e impedir a ocorrência a replicação viral (SWEVERS; LIU; SMAGGHE, 2018).

Essa técnica pode ser aplicada para terapias gênicas e para pesquisa científica. A terapia gênica pode ocorrer através da administração de sequências de dsRNAs que irão impedir a tradução e produção de proteínas relacionadas a doenças genéticas. O grande desafio dessa aplicabilidade é encontrar um vetor que seja eficiente e capaz de direcionar o dsRNA para células sem que o mesmo sofra degradação pelas enzimas degradadoras de RNAs (SETTEN; ROSSI; HAN, 2019; WENG et al., 2019).

Para a pesquisa científica é possível a produção de animais transgênicos modelos, como *D. melanogaster*, que carregam sequências em seu DNA que irão produzir dsRNA. E ainda, o uso do sistema UAS-GAL4 pode auxiliar nesse processo e permitir o *knockdown* genético em tecidos e estágios específicos (PERKINS et al., 2015).

Devido a importâncias dessas ferramentas o presente trabalho as utilizou para compreender a contribuição do gene CG15105 para o funcionamento do Sistema Nervoso. Utilizando a ferramenta de RNAi em *Drosophila melanogaster* para compreender como o *knockdown* de tais genes influência no funcionamento e morfologia cerebral. E por meio de análises de bioinformática busca compreender as interações moleculares relacionadas aos processos neuronais.

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CAPÍTULO 2- The knockdown of CG15105 on *Drosophila melanogaster* makes damage on brain and it has network with genes crucial to synaptic and cytoskeletal functions

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Abstract

Background: The CG15105 gene is neurodegeneration-related protein-encoding gene. The present study aimed to explore the pathophysiology effects of knockdown CG15015 on the brain *of Drosophila melanogaster* and the relationship between molecular interaction and neurodegeneration.

Methodology: Crosses were performed with transgenic *D. melanogaster* to obtain flies with dsRNA expression in the brain, submitted to the climbing test and histopathological analyses. In *silico analyses* were performed to understand how was the network of the interaction of one of the genes can act on neurodegeneration.

Results: The results showed that the knockdown of the CG15105 gene in the brain reduced the rate of climbing of flies to the ages evaluated. Significant histopathological changes were observed, there was presence of vacuoles on knockdown brain. The *in silico* analysis was performed to understand interaction network in motor deficit. The results showed that the gene encodes a protein called thin, which is orthologue to the human protein TRIM2 and has a binding site of the ubiquitin E3 and zinc finger (RING), and 46.03% of the genes in the interaction network of this protein are involved with ubiquitin E1 and E2. Thus, this protein may be related to the process of ubiquitination of proteins in which it establishes physical interaction, which is mostly proteins related to the cytoskeleton. This protein also has interaction with other proteins that perform functions in the response to hormonal stimuli and cycle and regulation of development. And finally, an analysis was made using brain transcriptome data from Alzheimer's model flies, and it was observed that transcriptional changes occur in some of the genes of the interaction network of the CG15105 gene, but until then such a relationship between these genes has been described only in muscle tissue.

Conclusion: The CG15105 is involved in brain development and functioning, and the knockdown causes a reduction of motor activity and morphological damage on brain..

Keywords: Neurodegeneration, TRIM, RNAi, ubiquitination.

1. Introduction

The CG15105 gene also called *tn,* has the function of connecting the ubiquitin-protein E3 and has the zinc finger domain. E3 proteins bind stems from binding to other proteins to mediate the ubiquitination process, whose function is to degrade proteins (DAHL-HALVARSSON et al., 2020). When ubiquitination processes are comprised change the proteolytic balance, this happened in neurodegenerative diseases, such as Alzheimer's disease (KURTISHI et al., 2019).

Studies show that the *TRIM2* gene plays a crucial function in the nervous system. Mutations in the *TRIM2* gene may lead to the development of axonal neuropathy (LI et al., 2020). In addition, the gene acts in the process of regulating neuron polarization through the process of ubiquitination of light chain filaments and has a key function during neurodevelopment (KHAZAEI et al., 2011).

A study developed by our research group showed that there is a reduction in the expression of the CG15105 on brain aging with the presence of β-amyloid accumulation using *Drosophila melanogaster* as an animal model (SILVA, 2019). Thus, the present study aims to revalidate the findings of our group and investigate the contributions of CG15105 to neurodegeneration, through the genetic knockdown of gene in the brain and *in silico* assay to understand molecular interactions and relation to Alzheimer disease.

2. Methods

2.1. Drosophila melanogaster

For the development of the present study, transgenic strains of *Drosophila melanogaster* were utilized, which were obtained by the *Drosophila* Bloomington Stock Center. They were kept in the Genetics Laboratory at the Federal University of Uberlândia a 25°C, with a 12h/12h light/dark cycle. They were fed ad libitum with standard Bloomington culture medium (soy powder 0,01%, glucose 7,2%, agar 0,6%, cornmeal 0,073%, yeast 0,018%, nipagin 0,06% and acid solution 0,05% m/v)

Females flies from the elav-Gal4 strain (BL#458) were mated with males of the w^{1118} (BL#3605) and UAS-dsRNACG1505 (BL#67287) strains to obtain negative control and knockdown flies for the CG15105 gene on brian to obtain flies with the genotypes of interest. The elav-Gal4 is a driver strain that will direct the expression of the responder to the brain of the flies, and the UAS-dsRNACG15105 are the responder strains that carry the dsRNA sequence for the mRNA of interest and will perform RNA interference and lead to knockdown of the selected genes. The w^{1118} strain is the mutant strain used as the genetic background for transgene construction, thus this strain was used to obtain flies from the negative control group that had only one copy of the drive transgene, and no responder.

Mating was made by anesthetizing flies with carbon dioxide. Female virgin flies - identified by visible meconium - were collected and then placed with male flies of the other strains. The offspring, having the genotype of interest, were collected 10 to 13 days after mating, to obtain flied ranging from 0h to 72h.

2.2. Locomotor activity

The behavior of flies was evaluated using the rapid interactive negative geotaxis test, which is based on the negative geotaxis behavior of *D. melanogaster* and can assess the locomotor ability of the flies. The performance of the flies in this test can be influenced by age and nervous system impairment. Thus, such as test allowed us to assess the influence of genes of interest on neurodegeneration over time (GARGANO et al., 2005).

Flies were placed in transparent tubes 9.5 cm high by 2.3 cm in diameter, placed in 12-seat racks, and positioned 40 cm away from a film camera and a fluorescent light.

The flies were left in the environment for 20 minutes, and then the pad was tapped three times, and the number of flies able to climb to a height of 5 cm within 4 seconds of the last tap of the pad was evaluated. Flies were submitted to this test at ages of 1-4, 3-6 and 8-11 days after eclosion (d.a.e.). The environmental conditions were controlled, the experiment was performed in a place with little noise, with an ambient temperature of 25°C ± 2, and always performed at similar times. Three tubes with 20 to 30 flies were used.

2.3. Histopathological analyses

Light microscopy was performed to evaluate morphological changes in the brain caused by the knockdown of the CG15105 gene. Ten adult flies were collected from each experimental group, and anesthetized on ice, and fixed with Carnoy's solution (6:3:1 of 99% ethanol, chloroform, and glacial acetic acid) with a solution volume of 10x the sample volume for 24 hours or more, and then the flies were decapitated and the heads were dehydrated with ethyl alcohol in increasing concentrations (70%, 80%, 90%, and 100%) for 15 minutes each. Then diaphanized in two xylols for 15 minutes each. And embedded in paraffin by passing through a liquid paraffin bath (57°C to 60°C) for 30 minutes, to make the paraffin blocks. The blocks were cut with a microtome to a thickness of approximately 4 μm. The selected sections were placed on glass slides and left to dry in an oven at 40°C for 12 hours.

The slides were then deparaffinized with two xylols baths (40 minutes each batch) and hydrated with ethyl alcohol in decreasing concentrations (100%, 90% 80%, and 70%) and then water, stained with Harris' hematoxylin and yellow eosin, dehydrated with ethyl alcohol in increasing concentrations (70%, 80%, 90%, and 100%). Then the slides were sealed with Teflon and coverslips. Images were captured using a light microscope (Leica) coupled with an image capture system (LAS EZ software) at 100x and 1000x magnification.

Analysis of the images was done using ImageJ software, to quantify the degree of neurodegeneration, for we used images at 1000x magnification of the eye lobe of the brain of the flies, the images were cut equally to exclude the intecellular spaces of the fly's brain, and leaving only the medullary region of the eye lobe. The total and proportional area occupied by nerve cells was then quantified. The intercellular space rate was

125 calculated according to the percentage of areas with gaps, that is, interstitial space,
126 vacuolar lesions, and fissures.

127 **2.4. *In silico* analyses**

128 The *in silico* tests were performed to understand the structure, function, and
129 interaction network of the protein encoded by the CG15105 gene. For this, databases and
130 software were used to obtain the desired information.

131 To understand the structure queries to the Uniprot, Swiss-model, and Interprot
132 databases were used. To evaluate protein function the Uniprot, Flybase, and NCBI
133 databases were used. Finally, to evaluate the protein interaction network, EsyN, MIST,
134 STRING, and ClueGO software were used.

135 In the Uniprot platform, there were data of five isoforms of the thin protein from
136 *D. melanogaster*, given the similarities of the information, isoform C
137 (B7YZK8_DROME) was used as a guide. The same isoform was considered for the
138 evaluation in Swiss-model and Interprot software.

139 Flybase information was obtained by analysis of the *tn* gene, whose id is
140 FBgn0265356 data obtained from version FB2021_03, released June 15, 2021.

141 For the analysis in the software MIST @ Harvard Medical School v5.0 (April
142 2020), the *D. melanogaster* species was used as a parameter, using a filter that removed
143 the lowest results in the rank.

144 In the analysis using the String database version 11.0 the results of the tn protein,
145 C isoforms from *D. melanogaster* were queried, and the results were not separated into
146 groups.

147 For the ClueGO analysis, version v2.5.8 was used. In this software, it was
148 evaluated which genes relate to the CG15105 gene and paired with the database generated
149 in the transcriptome analysis of COSTA-SILVA (2019), and a correlation of their
150 contributions to cellular processes was made.

151 **2.5. Statistical analyses**

152 Results were analyzed using Excel 2016 and GraphPad Prism 8 software. The
153 results of the scaling were analyzed by applying the D'Agostino-Pearson normality test
154 to assess whether the data had a Gaussian distribution, the results pointed out that all

groups were with normal distribution, then two-way ANOVA with Tukey's post hoc test was used to assess the statistically significant difference between the treated group and the control group. The histology results were analyzed by applying the D'Agostino-Pearson normality test and the results indicated that the data had a Gaussian (normal) distribution, and then T test was applied.

3. Results

3.1. The knockdown flies decrease the climb rate compared to control group

The knockdown flies of gene CG15105 (elav-GAL4/+; UAS-dsRNACG1505/+) show reduction of climb rate when it compared to control group (elav-GAL4/+; +/+). This reduction could be observed in flies with age 1 to 4 days after eclosion (d.a.e) p=0.0061, and age 3 to 6 d.a.e. (p=0.0014) and 8 to 11 d.a.e. (p=0.0271), Figure 1.

3.2. The knockdown of CG15105 causes morphological alterations on brain

The histopathological analysis shows presence of short and abundant vacuoles on knockdown flies' brain (figure2-a), and quantitative analyses on ImageJ show statically differences and increase of intercellular space (p=0.023) in flies' brains caused by the knockdown of gene CG15105 on age 8 to 11 d.a.e. (Figure 2-b).

3.3. Molecular network of gene CG15105 and the relation with aging and β-amyloid peptide

To understand how these genes, contribute to the neurodegenerative process we choose the gene CG15105 for make *in silico* analyses to realize how the network of this gene operates neuronal process.

The gene CG15105, also call by *tn*, encodes THIN protein (Figure 3). This protein is a Tripartite Motif (TRIM) and it has RING domain on N ends. RING's function is binding on zinc and makes transcription regulation. THIN, also, have an ubiquitin E3 ligase domain and there is homology with the six-bladed beta-propeller domain.

The data obtained with software Uniprot, Flybase and NCBI showed that this protein has activity on translation suppression, binding ubiquitin-protein, and zinc. THIN

plays a role in homeostasis and development of muscle cells, and positive regulation of the glycolytic process. The main location on body is at the Z disc on muscle cells.

After the structure and role analysis *in silico*, network analyses are performed to understand how this gene can be involved in the neuronal process. Then we observed a diversity network with different genes with varied roles (Table 1).

The software EsyN showed the CG15105 has a physical interaction with genes *ald1, tm2, mib2,* and *pglym78* (Supplementary Figure 1). Also, have a genetic interaction by transcription regulation. The genes *sallimus (sis)* and *estrogen-related receptor (err)* suppress *tn* gene, while α *actin* enhances *tn* expression (Supplementary Figure 2).

On the other hand, the *tn* gene could change the expression of other genes. It makes suppression expression of *muscle liim protein at 84b (mlp84p), pdgf- and vegf-receptor related (pvr)* and enhance expression of *cin85 and cd2ap related (cindr).* Moreover, *tn* could suppress and enhance the expression of *myosin heavy chain (mhc)*.

The software MIST could show a varied network of genes that results from predictions and data from others research (Supplementary Figure 3). It is possible to see that are genetic, proteic, and phenotypic interactions.

The thin protein has a physics interaction with proteins encodes by *pdha, semaphorin 1 a (sema1a), fas2,* and *spn*. These proteins have an important role in the development and function of the nervous system.

There is an expression relation between *tn* and genes*: l(2)efl, mlp84, lectin-galc1, cpn,* CG45078*, scla, sclb, mlc1, mp20, smyda-9,* CG15247*, prm, clect27,* CG5177, CG17107*, aqp, kah, zasp66, neurochondrin,* CG9626, CG9297, CG5023, *mtnc, tm2, up,* and *sals*. Some of these genes have roles specifics of muscle, while others have roles on carbohydrates metabolism, genetic regulation, membranes, metal binding, and embryological development, that contribute to neurological damage caused by tn knockdown.

Interologs interactions are protein interactions described in other species, and that this interaction can occur in orthologous proteins in the species in question due to the preservation of biochemical characteristics throughout evolution. The *tn* gene presents interolog interactions with proteins encoded by the *ubc2, brat, didum*, CG7220, *hsp83, ubc4, and eff* genes. The *brat* gene *(brain tumor)* encodes a tumor suppressor that

regulates the proliferation of cells in the brain. This protein inhibits the translation of proteins, prevents self-renewal, and induces differentiation during the process of dividing neural stem cells (EDWARDS et al., 2003).

CG15105 also establishes a phenotypic correlation with other genes, meaning that these genes are similar or phenotypically opposed, these genes may be part of the same protein complex or be involved in similar cellular processes. The genes that establish this correlation with tn are CG31224, *elF1A, Nup358, Rpt5, mts, tinc, wdb,* and *E(bx)* (Table 1).

The analyses on STRING software indicated some genes that had already appeared in the results of previous analyses, such as genes a*ctin, sls, mib2, mlp84B*, and other genes that does not had previously been observed, such as d*cr-1, dcr-2, lin-28, bub-1, ken and min* (Supplementary Figure 4).

After the analyses described so far, a list of all genes that interact with CG15105 and the encoders of proteins that interact with thin (Table 1), an analysis was made in ClueGO with the objective of understanding in which biological processes the genes in question acted (Figure 7). It was observed that 46.3% of the genes worked in the process of active ubiquitin synthesis, mainly E1 and E2, 23.81% of genes act in the process of contraction of muscle fibers, 7.94% act in the responses to hormonal stimuli, 6.35% has the function of negatively regulating cell development, and 6.35% are involved with muscle system functions, 3.17% regulate the JACK-STAT pathway that acts in signaling receptors. Other metabolic functions appeared in lower percentages, such as plasma mRNA concentration, pre-mRNA processing, pyruvate metabolism, and myoblast fusion.

To understand how the transcription of genes in this interaction network is affected in a brain by aging with and without the accumulation of the β-amyloid peptide, a comparison between data obtained with bioinformatics analyses and the results obtained in SILVA (2019) was performed. After pairing the data, it was observed that not all genes found in the predictions had their transcriptions altered throughout age, and none of the genes that interact with CG15105 have their transcription altered when comparing the control groups and β-amyloid group. Then, the genes whose transcription is altered over age in both groups were selected and this interaction was evaluated using ClueGO software.

It was observed that the genes *tn, prm, mp20, tm2, mlc1* and *mhc* had their transcriptions increased with aging without the presence of accumulation of the peptide β-amyloid, while the *l(2)efl* gene has reduced transcription with aging without the presence of accumulation of the β-amyloid peptide in *D. melanogaster* brains (Figure 6). Analyses using database information showed that the interaction between the genes mentioned above was described only in specific functions of the muscular system, and now we describe these alterations in *D. melanogaster* heads.

The genes *tn, prm, up, tm2,* and *mlc1* had their transcriptions increased with aging with the presence of accumulation of the β-amyloid peptide, while the l(2)efl gene has reduced transcription and aging with the presence of accumulation of the β-amyloid peptide in the brains of *D. melanogaster* (Figure 7). Analyses using database information showed that the interaction between the genes mentioned above was described only in specific functions of the muscular system, however this data showed these genes have alterations of transcription on heads.

We can observe that there is a difference in the transcription pattern of genes that interact with the *tn* gene throughout aging with and without accumulation of the β-amyloid peptide in *D. melanogaster* heads. The *tn, Prm, Tm2, Mlc1,* and *l(2)efl* genes follow a similar transcription pattern in both aging processes. However, the *mp20* and *mhc* genes have their expression reduced only in aging without the presence of accumulation of the β-amyloid peptide, while the gene up has its expression reduced only in aging with the presence of accumulation of the β-amyloid peptide.

4. Discussion

The knockdown on brain of the CG15105 gene caused a reduction in locomotor activity and did not cause significant motor changes. The results of the *in silico* analyses showed that the CG15105 gene and 46.3% of its interaction network are directly related to ubiquitin E1 and E2. Other functions also appeared in significant proportions such as muscle contraction process, hormonal stimuli, and cell development. These results suggest that knockdown CG15105 changes physiology of brain and maybe this process is because of ubiquitination.

Among the proteins that establish physical interaction with TRIM are some proteins that act in neurological processes, such as *pdha, mib2, fas2, sema1a,* and *spn*. And in this way, the *tn* protein may be involved with the ubiquitination process of these

proteins that perform plasticity and synaptic signaling functions. The *pdha* encodes a protein that has dehydrogenase pyruvate functions and contributes to acetyl-CoA synthesis (WISIDAGAMA; THUMMEL, 2019). And the *sema1a* encodes a protein transmembrane with the role to control dendrites and the axon direction, this gene is essential to motor neurons functions, and also, have an interation with the receptor encodes by *plexa* (HONG et al., 2020; JEONG, 2017). In this way, there is another one in this network involved in synaptic activity, it is *spn* that regulates the signal of neurexin and neuroligin, that active presynaptic zone (RAMESH et al., 2021). And the *fas2* encodes a protein that regulates nerve fascicles using molecular connexion with axon MP1 pathway and play roles on motor and memories formation (CHENG et al., 2001; KIM; WEN; JAN, 2009).

The TRIM2 protein was already with the function of ubiquitination of light chain neurofilament, which led to the contribution to neuronal polarization during neuronal development (KHAZAEI et al., 2011). Also, the deficient of TRIM2 cause to accumulation of light chain neurofilaments on brain, and this cause neurodegeneration, how we showed with climb test, the behavior can change (BALASTIK et al., 2008).

In addition, the TRIM2 protein is related to cellular plasticity in the early stages of neurological development, and suppression of the TRIM2 coding gene leads to cellular apoptosis (LOKAPALLY et al., 2020).

In the last analysis in which it was considerations, specific transcriptions of *Drosophilas* heads showed genes that until then had important interactions for muscle functions. This may have occurred because most of them are related to cytoskeletons, and the findings related to functions are more related to muscle contraction. However, our results suggest that these genes are associated with brain functions, and transcriptional changes are associated with neurodegeneration, aging, and motor deficit.

5. Conclusion

The *tn* (CG15105) gene is involved in genetic and biochemical processes related to brain development and functioning and has an interaction network capable of regulating different neurodegeneration-related cellular processes, such as ubiquity and synaptic regulation. And more studies are needed to track in more detail the genetic and protein changes caused by the knockdown of this gene, including in a pathophysiological situation of β-amyloid peptide accumulation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figures

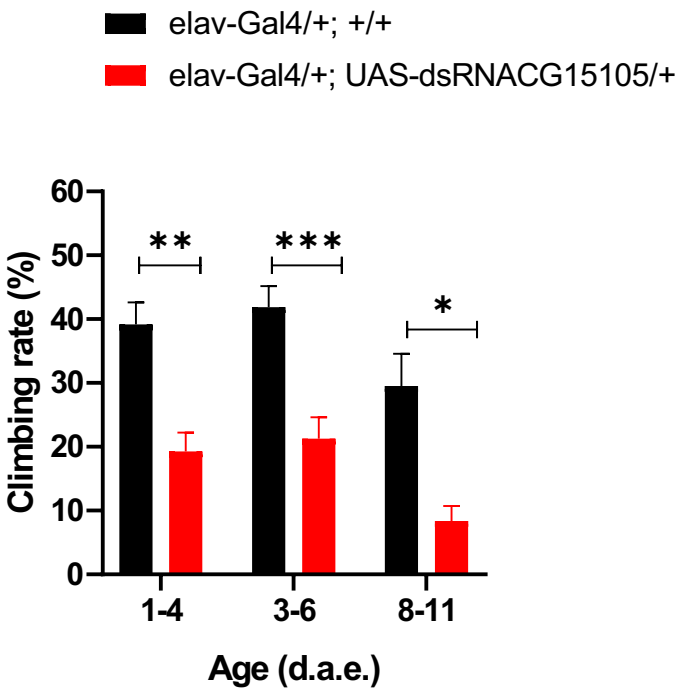
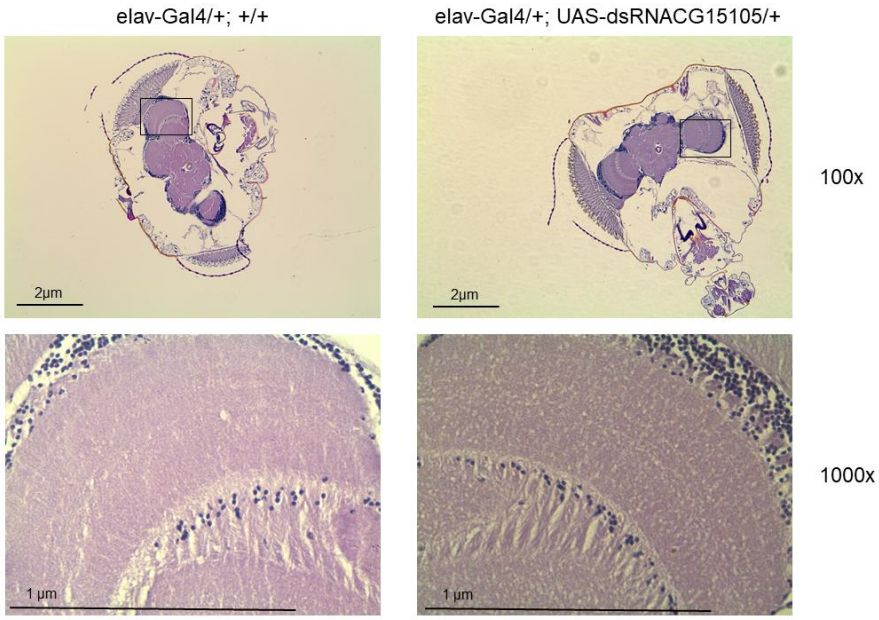


Figure 1- Evaluation of the climb rate in different days after the eclosion of knockdown flies for the CG15105 gene and negative control. Percentage of flies that climbed height equal to or greater than 5 cm after four seconds. Legend represents the genotype of the flies of each group. The bars represent the arithmetic mean ± standard error. Two-way ANOVA was applied with Tukey *post hoc* test to evaluate the significance of the difference of the knockdown group about the control in each age. It was represented with * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

A



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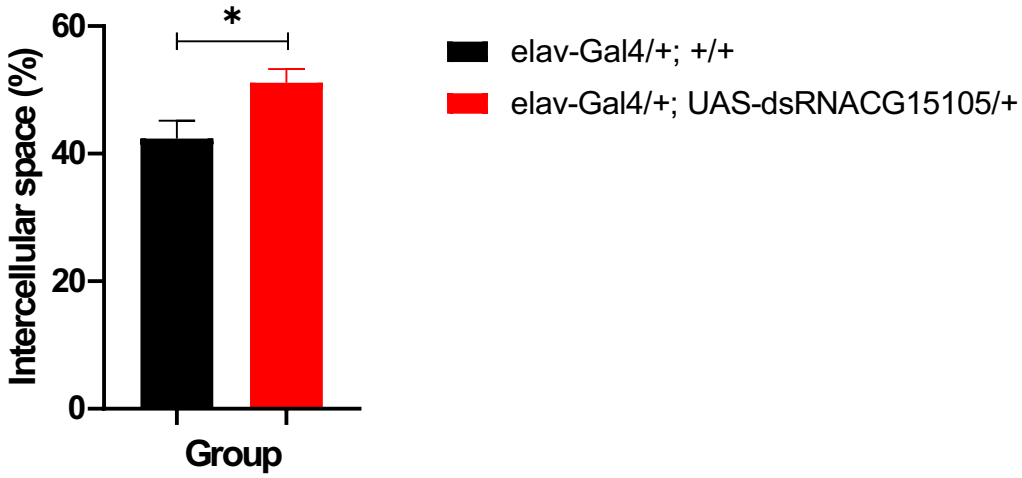


Figure 2- Evaluation of histopathological changes induced by knockdown of CG1505 genes in the brain of *D. melanogaster*. A- Images of histopathological slice of heads of *D. melanogaster* at 8 to 11 d.a.e.. Samples smeared with hematoxylin and eosin and images captured using Leica light microscope with the aid of LAS EZ software with an increase of 100x and 1000x.B- Graph representing the brain intercellular space of each group at the age of 8 to 11 days. The T test with was performed and found p=0.023.

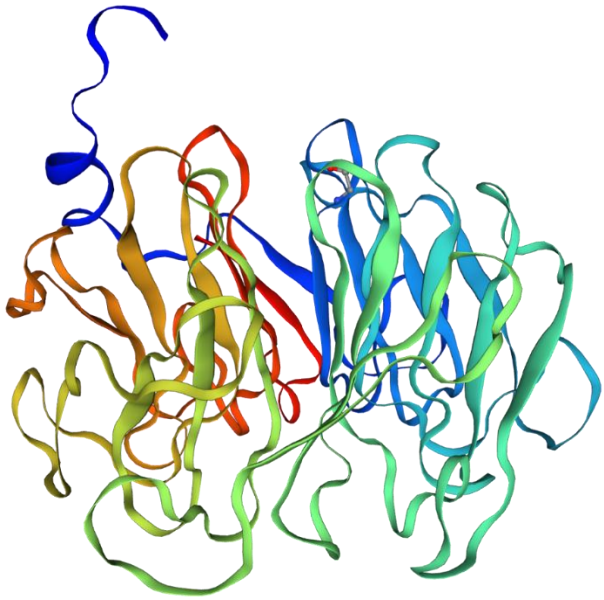


Figure 3 - 3D structure of the thin protein. Image from Uniprot

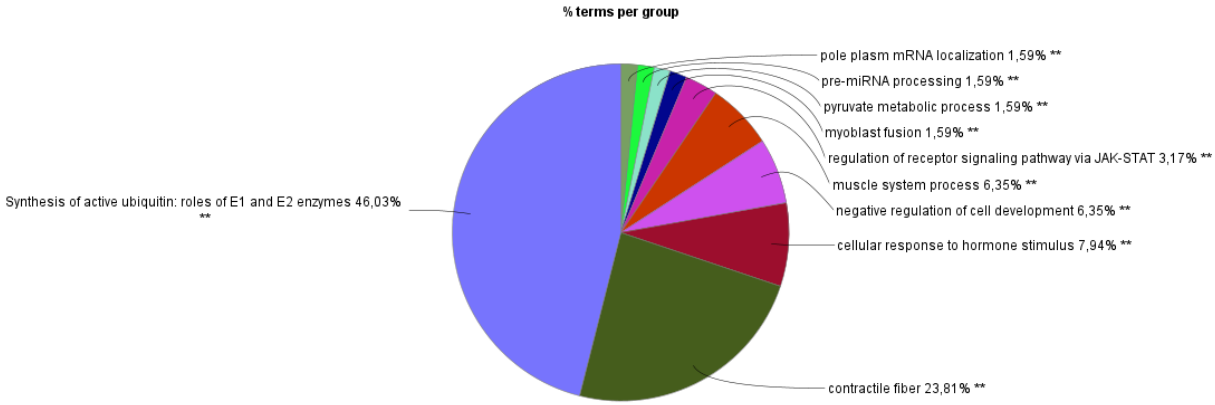
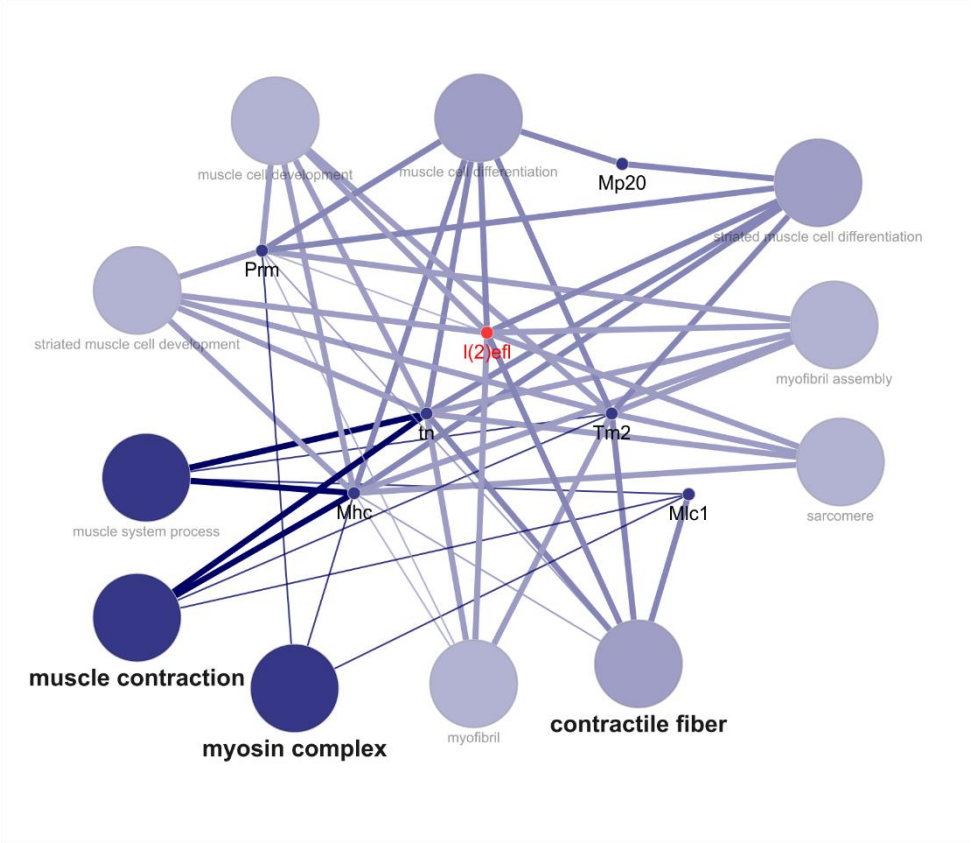


Figure 4- Functions of genes of CG15105 network. Image obtained with the analyses performed by the ClueGO bioinformatic tool.



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388 **Figure 5- Results of the analysis of the transcripts of the negative control**
389 **group with 0-3 d.a.e. and 10 d.a.e.** In blue are the genes that have increased their
390 transcription with aging and in red the gene that had its transcription reduced. This
391 network showed genes that are related to aging on brain and are on network on CG15105
392 gene, and the cell process that was descrite.

393

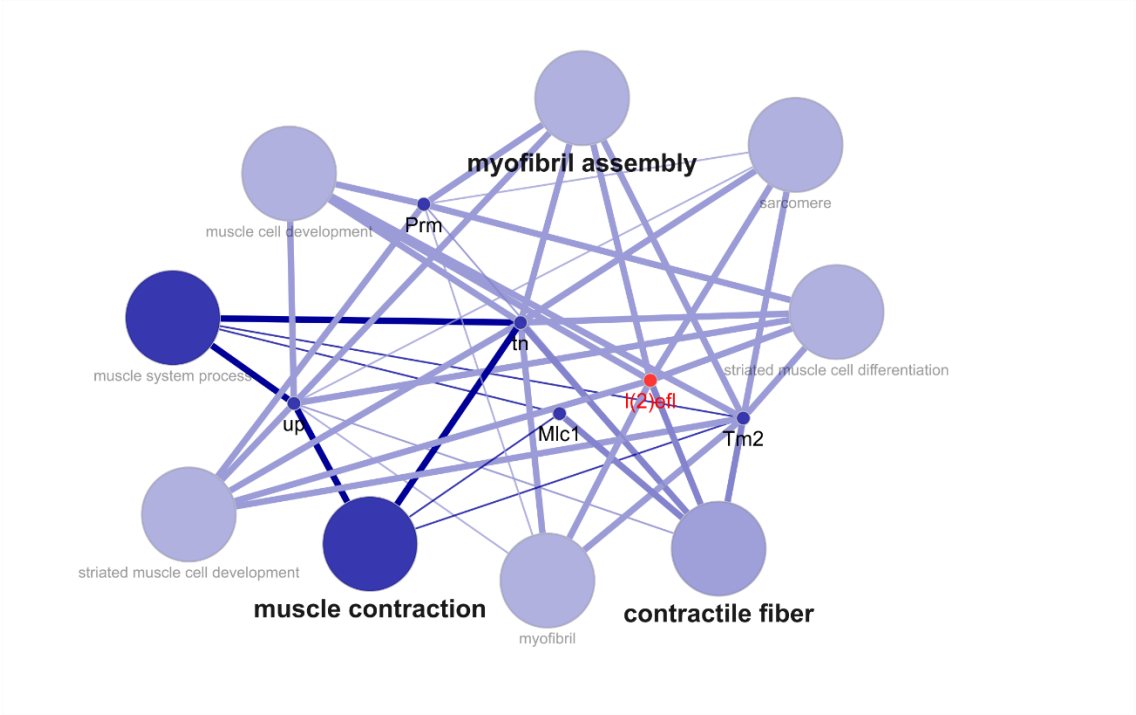

Figure 6- Results of the analysis of the transcripts of the group with the accumulation of β-amyloid with 0-3 d.a.e. and 10 d.a.e. In blue are the genes that have increased their transcription with aging and in red the gene that had its transcription reduced. This network showed genes that are related to aging an accumulation of β-amyloid on brain and are on network on CG15105 gene, and the cell process that was descrite.

403 **Table 1**

404 **Table of *tn* network-** Data from analyses and data bases of softwares: EsyN, MIST, STRING, FlyBase and NCBI.

REFERENCE	GENE	NAME	PROTEIN	INTERACTION WITH CG15105	FUNCTION
tn	CG15105	Thin	Tn	-	The protein of the TRIM/RBC family plays a role in the structure of myofibrils.
sls	CG1915	Sallimus	sls	Suppresses expression/ Co-expression	The protein binds to myosin filaments on the Z disc.
mhc	CG17927	Myosin Heavy chain	MHC	It has the expression regulated	Plays a role in muscle contraction.
actn	CG4376	α-actin	Actn	Increases expression/ Co-expression	Gene encodes specific actin proteins of muscle cells and actin from other tissues that form the cytoskeleton of the cell.
mlp84b	CG1019	Muscle LIM protein at 84B	MLP84B	It has the expression suppress/ Coexpression	Encodes a protein that makes up the cytoskeleton of muscle cells.
ald1	CG6058	Aldolase 1	Ald1	Physics	Encodes an enzyme that acts on two targets, fructose-1,6-

					biphosphatase and fructose-1-phosphatase
tm2	CG4843	Tropomyosin 2	TM2	Physics	It is associated with the troponin complex and plays an important role in regulating calcium-dependent muscle contraction.
mib2	CG17492	Mind Bomb 2	Mib2	Physics/ Co-expression	Encodes a protein that acts on synaptic plasticity and muscle integrity.
pglym78	CG1721	Phosphoglyceromutase 78	pglym78	Physics	Encodes an enzyme that performs activities in the fusion of myofibers and the somatic development of muscle cells.
fas2	CG3665	Fasciclin 2	Fas2	Physical interaction	It is involved with the development of the nerve fascicle, through molecular reconnection with the MP1 pathway of the axon.
pdha	CG7010	Pyruvate dehydrogenase E1 alpha subunit	Pdha	Physical interaction	Pyruvate dehydrogenase activity, more specifically acetyl-transferase, and participates in the biosynthesis of acetyl-CoA

sema1a	CG18405	Semaphorin 1a	sema1a	Physical interaction	It is a transmembrane protein and plays a receptor role that regulates the target of dendrites and the direction of axons. It also establishes interactions with the receiver encoded by PlexA.
spn	CG16757	Spinophilin	Spn	Physical interaction	It is a protein that regulates neurixin/neuroligin signaling that activates the pre-sinaptic zone.
l(2)efl	CG4533	lethal (2) essential for life	L(2)EFL	Expression	It has a vital function during embryological development
lectin-galc1	CG9976	Galactose-specific C-type lectin	Lectin-galC1	Expression	Encodes a protein, it binding to galactose and is involved with cell-cell afore.
cpn	CG4795	Calphotin	Cpn	Expression	It has ca^{2+} buffer function in photoreceptor microvilli, this occurs due to strong negative charge, and protein composition rich in glutamic acid and highly hydrophobic amino

					acids. With this, calphotin protects photoreceptor cells from Ca^{2+} overload and degeneration caused by light.
CG45076	CG45076	No information	No information	Expression	It is involved with the transport of proteins, it has been predicted that it may be present in the plasma membrane and is expressed in the head and heart in adult flies.
CG45078	CG45078	No information	No information	Expression	Encodes a protein and is expressed in the head and heart in adult flies.
scla	CG45090	Sarcolamban A	SclA	Expression	Encodes a protein that plays a central role in regulating calcium transport in the sarcoplasmic reticulum and regulating muscle contraction.
sclb	CG45091	Sarcolamban B	SclB	Expression	Encodes a protein that plays a central role in regulating calcium transport in the sarcoplasmic reticulum and regulating muscle contraction.

mlc1	CG5596	Myosin alkali light chain	Mlc1	Expression	Encodes a protein that binds to calcium ions located in the cytosol and acts on the development of mesoderm. It's part of the myosin complex of muscle cells.
mp20	CG4696	Muscle protein 20	Mp20	Expression	Encodes a protein that acts in the regulation of myoblast fusion. And it can be found in the cytoplasm.
smyda-9	CG12119	SET and MYND domain-containing, arthropod-specific, member 9	SmydA-9	Expression	It binding activity to histone deacetylase and lysine-N-methyltransferase protein, which negatively regulate gene expression
CG15247	CG15247	No information	No information	Expression	Encodes a protein, the expression was observed in the embryonic, larval and adult male stages. No more information about its expression and function.
prm	CG5939	Paramyosin	Prm	Expression	Encodes a specific structural protein of muscle cells of

					invertebrates, can phosphorylate and this acts on an important muscle function.
clect27	CG3244	Schlaff	SLF	Expression	Encodes a chitin-binding protein that is involved in the formation of the asa.
CG5177	CG5177	enzyme Trehalose-phosphatase	Trehalose 6-phosphate phosphatase	Expression	Participates in the process of biosynthesis of trehalose, is located in the extracellular medium, and is expressed in adult heads and other organs of the fly body.
CG17107	CG17107	No information	No information	Expression	The gene encodes a protein that is expressed in the middle intestine in the embryonic and larval stages.
aqp	CG12251	aquaporin	AQP	Expression	Encodes a protein that has channel function, is active in cytoplasmic, and is expressed in adult fly heads.
kah	CG17181	Kahuli	Kah	Expression	Regulates transcription by connecting to E-box

zasp66	CG6416	Z band alternatively spliced PDZ-motif protein 66	Zasp66	Expression	Encodes that protein that binds actin, is located in the Z disk of the muscle fiber.
neurochondrin	CG2330	Neurochondrin	Neurochondrin	Expression	It has the role of up-regulation of the MHC, is involved in the process of regulation of synaptic plasticity and development of neurons.
CG9626	CG9626	No information	No information	Expression	No information
CG9297	CG9297	No information	No information	Expression	Encodes a protein that is involved with the process of endocytosis and transport by binding to organelle membranes and the plasma membrane.
CG5023	CG5023	No information	No information	Expression	It binds to actin and has the function of organizing the actinomyosin complex and is active in the cytoskeleton.
mtnc	CG5097	Metallothionein C	MtnC	Expression	It connected to metal ions
up	CG7107	upheld	Troponin T	Expression	It encodes a protein that regulates tropomyosin and is found to thin

					filaments in the muscle sarcomere.
sals	CG31374	sarcomere length short	Sals	Expression	Encodes a protein that binds actin and acts on the growth of muscle fiber.
ubc2	CG6720	Ubiquitin-conjugating enzyme 2	Ubc2	Interolog	Catalyze the covalent binding of ubiquitin to other proteins
brat	CG10719	brain tumor	Brat	Interolog	Encodes a tumor suppressor that regulates the proliferation of cells in the brain. This protein inhibits the translation of proteins, prevents self-renewal, and induces differentiation during the process of dividing neural stem cells.
didum	CG2146	dilute class unconventional myosin	Didum	Interolog	It has mitochondrial transport function in neuronal cells.
CG7220	CG7220	CG7220	Conjugate enzyme ubiquitin E2	Interolog	It is involved with the activity of proteases that mediate the process of protein catabolism, monoubiquitination, and protein polyubiquitination.

hsp83	CG1242	Heat shock protein 83	HSP83	Interolog	Encodes chaperones that promote maturation, structural maintenance, and regulation of the properties of specific proteins that have the function of controlling the cell cycle. The gene goes through a functional cycle that is linked to ATPase activity, this cycle induces conformational changes in proteins leading to its activation. And it interacts dynamically with co-chaperones that recognize the substrate, ATPase cycle, and functional chaperones. Together with Hop and piwi, they support the channelization and development via epigenetic silencing of genetic variants and suppression of genetic variants induced by transposons. Required by

					piRNA biogenesis and facilitated the loading of piRNA inside piwi proteins.
ubc4	CG8284	Ubiquitin-conjugating enzyme 4	UBC4	Interolog	Catalyze the covalent binding of ubiquitin to other proteins
eff	CG7425	Effete	Eff	Interolog	Encodes the conserved part of the enzymes conjugated to ubiquitin E2 class I which functions of ubiquitination and degradation and is related to the regulation of apoptosis and chromatin organization.
rpt5	CG10370	Regulatory particle triple-A ATPase 5	Rpt5	phenotypic correlation	Encodes a component of proteasome 26S, which degrades polyubiquitinated proteins in the cytoplasm and nucleus.
tinc	CG31247	tincar	Tinc	phenotypic correlation	Encodes a transmembrane protein that is involved with regulating the development of cellular omatides.

e(bx)	CG32346	Enhancer of bithorax	E(bx)	phenotypic correlation	It is a component of binding to hist*one remodeling factor (NURF),* which is an ATP-dependent catalysis complex of nucleosome slippage and transcription facilitator
elf1a	CG8280	Elongation factor 1 alpha	ElF1A	phenotypic correlation	Encodes proteins necessary for mRNA transcription, is involved with the 43S pre-initiation complex, ensuring the selection of the aug codon start and its dissociation by the ribosome subunit 40S.
nup358	CG11856	Nucleoporin 358kD	Nup358	phenotypic correlation	Encodes a protein that makes up the nuclear pore complex, which regulates transport through the nuclear envelope and plays a crucial role in the transport of mRNA by recruiting the mRNA transport complex composed of Nxt1 and sbr/Nxf1

wdb	CG5643	widerborst	wdb	phenotypic correlation	Encodes a subunit B' regulatory of the serine/threonine phosphatase PP2A complex, which regulates Hedgehog, a protein kinase B, and the insulin receptor signaling pathway.
mts	CG7109	star microtubule	mts	phenotypic correlation	Encodes the a2 phosphatase subunit, which is involved in various development processes and signaling pathways.
CG31224	CG31224	No information	No information	phenotypic correlation	It is a protein-coding gene that has the function of linking to DNA and suppressing transcription. It is involved with the regulation of transcription by RNA polymerase II.
bub2	CG14030	Bub1 kinase	Bub2	Co-expression	Encodes a protein that participates in a checkpoint and is involved with the formation of the

					cytolytic spindle and the endocytosis of parasites.
dcr-1	CG4792	Dicer-1	Dcr-1	Co-expression	It encodes an enzyme that participates in the RNA interference process, the enzyme catalyzes the cleavage of dsRNA.
dcr-2	CG6493	Dicer-2	Dcr-2	Co-expression	It encodes an enzyme that participates in the RNA interference process, the enzyme catalyzes the cleavage of dsRNA, specifically cleave viral RNA.
ken	CG5575	ken and barbie	Ken	Co-expression	Encodes a protein that is a transcription factor and participates in the process of genital formation.
lin-28	CG17334	Lin-28	Lin-28	Co-expression	Encodes an RNA-binding protein that participates in the micro RNA maturation process.
me	CG33558	missing-in-metastasis	me	Textmining	Encodes a protein involved in guided cell migration by inhibiting the endocytosis of the

		product encoded by Cortactin.

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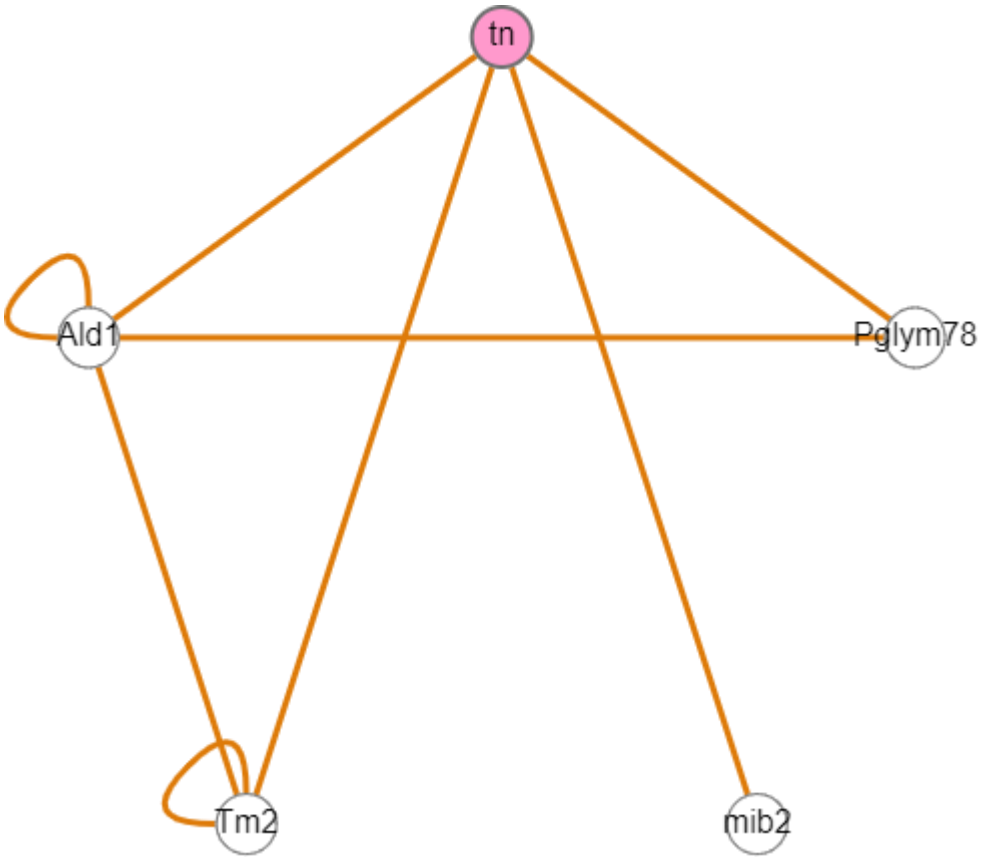
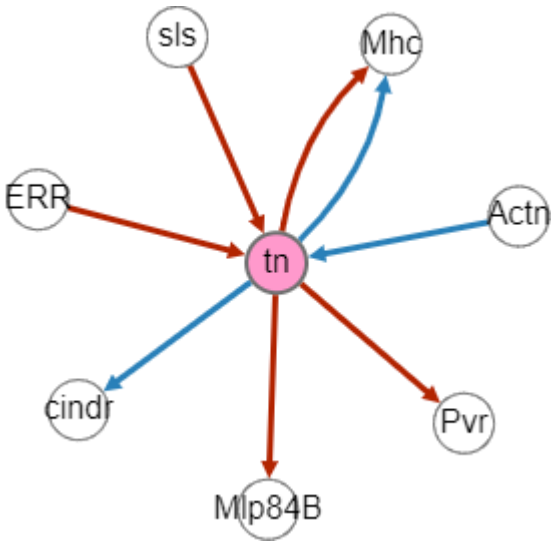
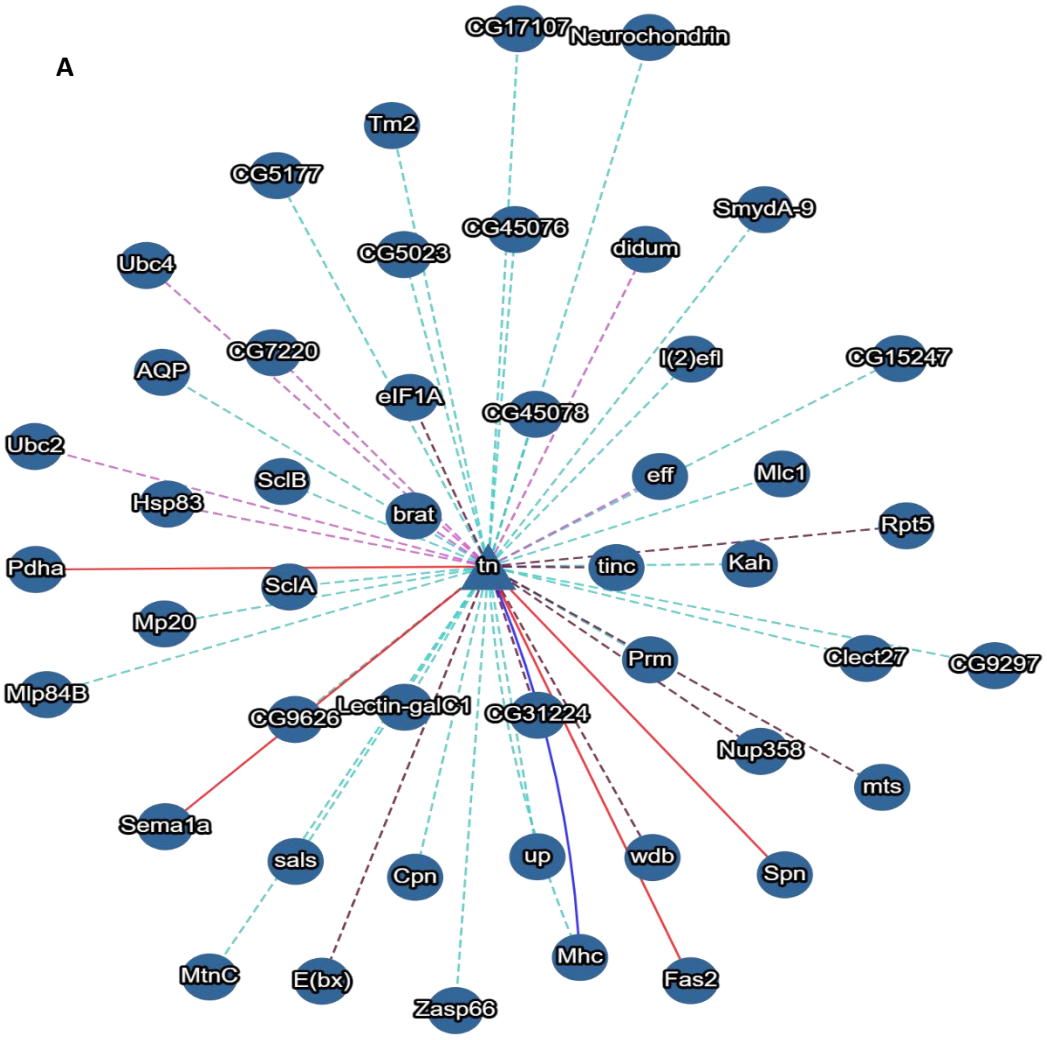
Supplementary figures



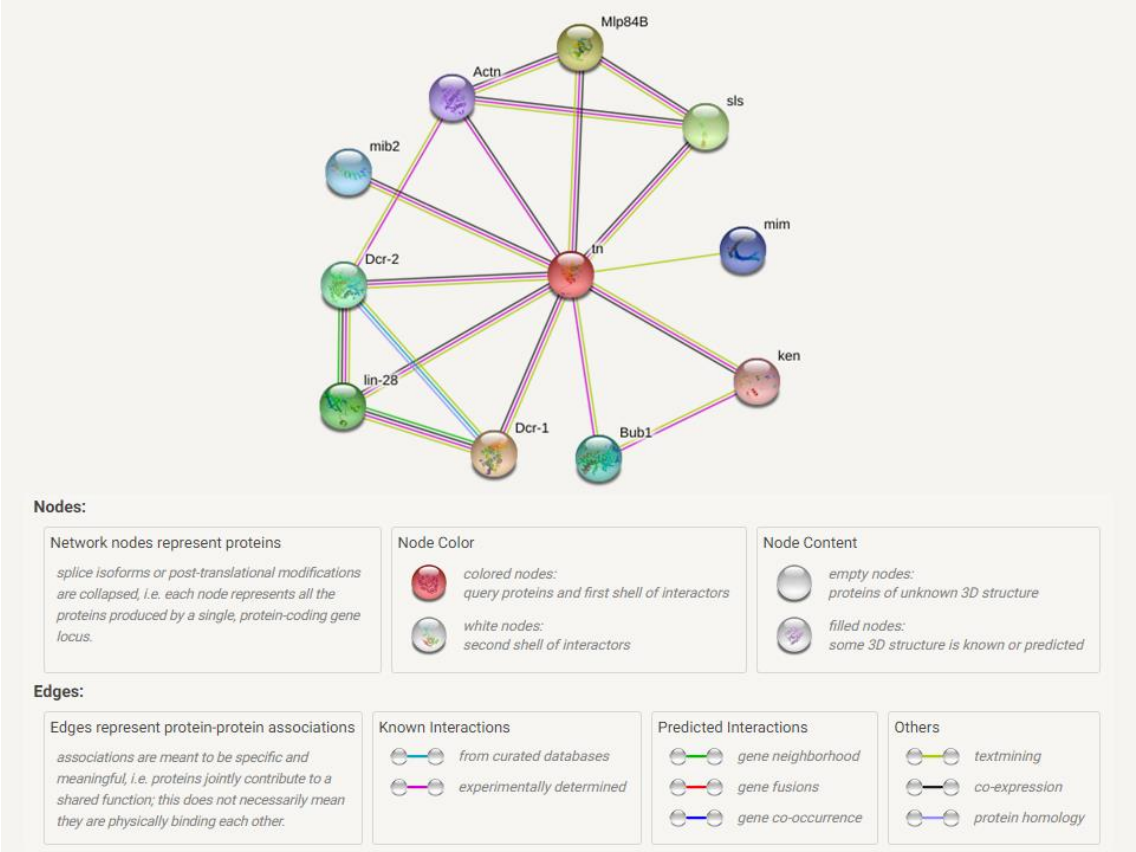
Supplementary Figure 1- Physical interaction network. Software esyN.



Supplementary Figure 2 – Genetic interaction network. Software esyN. Red arrow represents suppression of expression and blue arrow represents increased expression.



Supplementary Figure 3 – Interactions with the thin protein, represented with tn. The red solid line represents the protein-protein interactions, the blue solid line represents the genetic interactions, the blue, green, light purple and dark dotted lines represent the expression correlation interactions, interlog genetics, interlogs, and phenotypic correlations, respectively. Software MIST



Supplementary Figure 4 - Protein-protein associations. Software STRING