A role for SKN-1/NRF2 antioxidant transcription factor in *Caenorhabditis elegans* immunity

Ph.D. thesis

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INTRODUCTION

Adaptation to the changing environment is essential for survival. Basic physiological processes are associated with the continuous production of reactive metabolites including reactive oxygen species (ROS). My Ph.D. studies focused on the oxidative-xenobiotic stress response master regulator SKN-1/NRF2 transcription factor. In response to exogenous or endogenous ROS, NRF2 activates the expression of antioxidant and xenobiotic enzymes, which confers protection against oxidative stress and damages. Increased oxidative stress appears to play a role in various human conditions including aging and in several diseases, such as atherosclerosis, Alzheimer's disease, chronic obstructive pulmonary disease. The protective role of NRF2 in these diseases has already been described. Studying NRF2 interactors may shed light on its complex biological functions which further promote its recognition as a target in drug development.

Although NRF2 is an intensively studied protein, bioinformatic databases contain only a few NRF2 interactors. During my Ph.D. I have browsed the NRF2 literature to create a new database, which contains experimentally proven regulators of NRF2 with further information about the interactions. The database revealed a multifunctional character of NRF2. I also investigated the role of NRF2/SKN-1 in the immunity of *Caenorhabditis elegans.* This versatile model organism of innate immunity is also ubiquitously employed in aging and stress research, in which our lab has great traditions and we intended to study SKN-1 also in these fields.

Both NRF2 and its *C. elegans* ortholog, SKN-1 have fundamental role in the coordination of oxidative and xenobiotic stress responses. Both proteins are precisely regulated by phosphorylation. Among the regulators we find the kinases of major signaling pathways involved in the regulation of immunity: the p38 MAPK (activation) and the insulin/IGF (inhibition) pathways. This suggests that SKN-1 may play a role in the innate immune response. During intestinal infection, the immune signaling pathways induce the expression of antimicrobial proteins. Intestinal cells also produce ROS to kill pathogenic bacteria. Bacterial toxins and ROS production together create an oxidative environment which challenges the intestinal cells. Therefore, I investigated, whether SKN-1 contributes to the pathogen resistance of *C. elegans*.

Immunosenescence, decline in immunity during aging is universal in living organisms. In *C. elegans* a decrease in p38 MAPK signaling and intestinal tissue deterioration were described as major causes of immunosenescence. As the p38 MAPK pathway is a key activator of SKN-1, I hypothesized that SKN-1 induction upon infection could be impaired in aged worms. In humans the role of NRF2 in immunosenescence was not discovered yet, so my results in the *C. elegans* can open new directions in this field.

AIMS

My Ph.D. work was centered around the bioinformatic and experimental analysis of the potential biological, including immune-related functions of the NRF2/SKN-1 transcription factor. .

1. Major aims of the bioinformatic analysis of NRF2:

- To create a novel database, which contains the interactors of the mammalian NRF2 by manual curation.

- Determination and prediction of functions of the mammalian NRF2 based on the analysis of its interactors.

2. Major aims of studying SKN-1 in *C. elegans* model organism:

- Characterization of the role of SKN-1 in pathogen resistance in *C. elegans*.

- Analysis of the role of SKN-1 in immunosenescence in *C. elegans*.

METHODS

Bioinformatic methods to analyze NRF2 interactors and functions

Building the NRF2 interaction database

The NRF2 interaction database lists proteins, which interact with mammalian NRF2. The NRF2 interactor candidates were collected manually by using iHOP web service, which searches for keywords in Pubmed literature resource. Those publications were selected in which the NRF2 was studied in a mammalian species. For collecting data about the interaction, I have used a protocol previously established for building the SignaLink pathway resource in the ELTE Dept of Genetics. The NRF2 interaction database also contains information about the characteristics of the interaction: effect (activation/inhibition), direction, etc. I have repeated the same protocol for collecting KEAP1 interactors because KEAP1 is the most important regulator of NRF2. Each interaction is documented with the Pubmed ID of the publication reporting the verifying experiment(s). The manual curation was closed in November 2011.

Analysis of the functions of NRF2 interacting proteins

I used the GOTermFinder tool to map and statistically analyze the biological functions of NRF2 interactors. Those Gene Ontology (GO) functions were used for the analysis, which were found for more than 10 NRF2 partners. To determine new NRF2 functions I have compared the list of partner functions with the list of NRF2 functions.

Methods to study SKN-1 in Caenorhabditis elegans immunity

Maintenance of *Caenorhabditis elegans* strains

Nematodes were maintained on NGM (Nematode Growth Medium) at 15^{o}C or 20^{o}C. Plates were seeded with *Escherichia coli* OP50 strain, which served as food source for the worms.

Crossing *C. elegans* strains

To investigate the regulation of SKN-1 activation upon *P. aeruginosa* infection, the *Pgcs-1::gfp;pmk-1(km25)* és *Pgcs-1::gfp;tir-1(qd4)* strains were created by Crossing hermaphrodite *Pgcs-1::gfp* worms with *pmk-1(km25)* or *tir-1(qd4)* heterozygous male animals. Progeny was first selected for roller (*rol-6*) phenotype of the *Pgcs-1::gfp* strain. F2 generation was tested for homozygous *pmk-1(km25)* or *tir-1(qd4)* mutations (deletion alleles) by PCR. F3 generation was selected for homozygotic roller phenotype and re-tested for *pmk-1(km25)* or *tir-1(qd4)* mutations.

Gene silencing in *C. elegans* by feeding RNAi method

Gene silencing by feeding RNA interference is a popular method in *C. elegans* research. *E. coli* HT115(DE3) bacteria strain producing dsRNA grew overnight (ON) in LB medium supplemented with 100 μg/ml ampicillin. The ON bacteria culture was seeded onto NGM plates supplemented with 100 μg/ml ampicillin, 12.5 μg/ml tetracyclin and 1 mM IPTG (isopropyl β-D-1-thiogalactopyranoside). In case of using several RNAi bacteria strains, equal volume of the cultures was seeded in all condition. As a control, empty vector expressing HT115(DE3) bacteria was used in all RNAi experiment.

Killing assay

The pathogen resistance of different *C. elegans* strains were compared by measuring the survival of animals on pathogenic bacteria. Sterile hermaphrodites were used in killing assays to avoid 'bag of worms' phenotype, which causes the animals' early death. Silencing the *cdc-25.1* gene, which is a key factor in early stages of development, prevented progeny formation. For the killing assays nematodes grew on *cdc-25.1(RNAi)* plates till young adulthood at 20^{o}C. The worms for the experiments with *daf-2(e1370)* mutant animals developed at 15^{o}C till young adulthood. For examining the effect of aging on pathogen resistance, animals were placed onto OP50-NGM plates at 20^{o}C after reaching young adulthood till the killing assay. Two human opportunistic bacteria strains were used in killing assays to test the animals' immunity: the Gram-negative *Pseudomonas aeruginosa* PA14 and the Gram-positive

Enterococcus faecalis SdB262 strain. The survival of 30-30 young adults was measured on *E. faecalis* or *P. aeruginosa* bacteria lawn at 25°C, and at least 3 parallel plates were used in all conditions. Dead animals were scored every 12 hours till the extinction of the population.

Measurement of SKN-1 target gene activation by fluorescence microscopy

Expression of two SKN-1 target genes: *gcs-1* (γ-glutamine cysteine synthetase) and *gst-4* (glutathione-S-transferase) was studied by measuring the fluorescence of GFP (green fluorescence protein) fusion reporter transgenic strains. Fluorescence of the P*gcs-1::*GFP strain reflects the activation of the *gcs-1* promoter, while using *gst-4::gfp* the expression of GST-4 protein is followed. L3 larvae were exposed to *P. aeruginosa* PA14 or control OP50 bacteria for 24 h at 25°C. 30-60 animals were immobilized by 40 mM levamisole in M9 buffer on 2% agarose. Images were captured by DFC480 camera on Leica DMI6000B microscope in the Institute of Physiology, Semmelweis University.

Investigation of SKN-1 localization by fluorescence microscopy

Activated SKN-1 translocates from cytoplasm to nucleus to induce target gene expression. To follow SKN-1 localization *skn-1::gfp* transgen strain was studied. L3 larvae were exposed to *P. aeruginosa* PA14 or control OP50 bacteria for 5 h at 25°C. Images of at least 15 worms were taken as described earlier. Intestinal SKN-1::GFP positive nuclei were counted in each worm and compared in different strains and conditions. Representative pictures of nematodes were captured by a Zeiss LSM510 confocal laser scanning microscope equipped with a 40×/1.3 oil immersion objective (Plan-Neofluar, Zeiss) in the Institute of Physiology, Semmelweis University.

Killing assay with oxidative preconditioning

Effect of oxidative stress on pathogen resistance in *C. elegans* was not studied before. To establish the protocol 2d adults were treated with 0 mM (control) 1 mM, 1,5 mM and 2 mM H_2O_2 in liquid NGM for 2 h at 20°C. After oxidative pre-treatment, animals were placed onto OP50-NGM plates for a 12 h recovery period, then survival of nematodes were measured on *P. aeruginosa* PA14 plates. As 2 mM H_2O_2 concentration was found to be the most efficient, this concentration was used in later experiments.

Measurement of oxidative tolerance

Animals were grown on *cdc-25.1(RNAi)* with empty vector, *skn-1(RNAi)* or *wdr-23(RNAi)* plates till young adulthood. 30-35 worms were placed into liquid NGM supplemented with 3 mM or 5 mM H_2O_2 for 1 h at 20°C. After oxidative treatment, nematodes were incubated on OP50-NGM plates for 24 h at 20°C and dead worms were scored.

Measurement of *C. elegans* lifespan

Animals were grew on *cdc-25.1(RNAi)* till young adulthood at 20°C. 25-25 animals were placed onto OP50-NGM plates and at least 2 parallel plates were used in all conditions. Lifespan was measured at 25°C. Dead worms were scored each day till the extinction of the population..

Characterization of age-dependent SKN-1 target genes

To analyze age-dependent SKN-1 target genes, the overlapping genes of 3 published microarray databases were determined. 46 SKN-1 regulated genes were found among 379 genes exhibiting the most significant age-dependent decline in their expression (>10-fold at d6 *vs.* d15). Regulation of the expression of the identified genes was analyzed based on Wormbase data, focusing on PA14-, oxidative stress- or PMK-1-dependent regulation.

Statistical analysis

Data were analyzed by using the SPSS software 15.0. Survival curves were compared by Kaplan-Meyer log-rank test. To compare the means of several assays variables were analyzed by one-way ANOVA test. Results are expressed as mean ± standard deviation (SD). Statistical significance was indicated as follows: * $p<0.05$, ** $p<0.001$, *** $p<0.0001$.

RESULTS

Investigation of NRF2 functions by bioinformatic methods

NRF2 interaction database

Proteins that were reported to interact with NRF2 were collected manually by using iHOP, Pubmed and Chilibot web sources. The developed database contains 108 proteins, 131 directed and 15 undirected interactions. 42 % (55) of the directed interactions are inhibitions, while 58 % (76) is activation. The biochemical mechanism of the direct interactions was also integrated if it was available. Majority of the biochemical mechanisms were dimerization and phosphorylation.

Prediction of NRF2 functions by analyzing the interacting partners

To reveal the role of NRF2 in biological processes GO functions of NRF2 interacting partners were examined. The analysis showed that more than 30% of the interacting partners are multifunctional proteins as they are involved in several processes. Eight main biological functions were identified, from which five processes contained the same 30-35 interacting partners. These five processes were: signal transduction, stress, response to chemical stimuli, metabolism and development. The role of NRF2 was known in these biological processes except for development. The other three functional groups contained less proteins and overlaps with the previously introduced five processes. Immunity (27 proteins), reproduction (15 proteins) and wound-healing (16 proteins) have not been described among NRF2 GO functions before.

Investigation of the role of SKN-1 in C. elegans immunity

SKN-1 is required for immunity in *C. elegans*

To study the impact of SKN-1 to immunity, animals' survival on pathogenic bacteria were compared in absence and presence of SKN-1. *skn-1(zu135)* mutant animals served as null mutants as this allele contains an early STOP codon, which blocks the expression of all three isoforms of SKN-1. Survival of *skn-1(zu135)* mutant nematodes were measured on both Gram-negative *Pseudomonas aeruginosa* PA14 and

Gram-positive *Enterococcus faecalis* SdB262 strains. The *skn-1(zu135)* young adult animals exhibited significantly shorter lifespan compared to wild type N2 strain on *P. aeruginosa* PA14 bacteria ($p<0.0001$). Silencing *skn-1* gene by RNAi also resulted in shorter lifespan on PA14 bacteria compared to worms fed by empty vector expressing bacteria ($p<0.0001$). Survival of 2d adult *skn-1(RNAi)* treated animals and *skn-1* nullmutant nematodes also exhibited shorter lifespan on PA14 and *E. faecalis* SdB262 strain ($p<0.0001$).

P. aeruginosa infection triggers SKN-1 activation

Activated SKN-1 translocates to the nucleus. To investigate if SKN-1 nuclear translocation occurs upon *P. aeruginosa* PA14 exposure, *skn-1::gfp* L3 larvae were incubated on PA14 lawn for 5 hours. A massive accumulation of SKN-1::GFP in intestinal nuclei of infected larvae was detectable, compared to control animals fed by the non-pathogenic OP50 *E. coli* strain ($p<0.0001$). The specificity of this response was demonstrated by a complete inhibition using a *skn-1*-specific double-stranded RNA. To reveal a SKN-1-dependent transcriptional activation upon PA14 infection, *Pgcs-1::gfp* and g*st-4::gfp* L3 larvae were exposed to *P. aeruginosa* or *E. coli* OP50 for 24 h. While there is no detectable intestinal GFP in the animals incubated on OP50, upon PA14 infection expression of both SKN-1 target gene reporters were found in >60% of the population ($p<0.0001$). Both the *gcs-1* promoter activation and the GST-4 expression were significantly suppressed by feeding worms with *skn-1(RNAi)*, indicating the specific requirement of SKN-1 to elicit these responses. Thus, PA14 infection induces nuclear translocation of SKN-1 and transcriptional activation of its targets.

The TIR-1/PMK-1 pathway controls SKN-1 activation upon *P. aeruginosa* infection

The p38 MAPK ortholog PMK-1 has a fundamental role in *C. elegans* innate immunity. To reveal the regulation of SKN-1 upon *P. aeruginosa* infection, first the role of PMK-1 was investigated, as PMK-1 is a key factor in SKN-1 activation upon oxidative stress response. Expression of *Pgcs-1*::GFP reporter was monitored in a wild-type and a *pmk-1(km25)* null mutant genetic background. In absence of PMK-1 the

SKN-1-dependent activation of *gcs-1* was entirely prevented in response to PA14 infection ($p<0.0001$).

TIR-1 is a conserved Toll/IL-1 resistance (TIR) domain protein known to activate p38 MAPK signaling independently of the Toll-like receptor ortholog *tol-1* during PA14 infection. To study the impact of TIR-1 in SKN-1 activation, expression of *Pgcs-1*::GFP reporter was compared in *tir-1(RNAi)* treated and control (fed by empty vector producing bacteria) animals. Depletion of TIR-1 by RNAi prevented *Pgcs-1*::GFP fluorescence upon PA14 infection. Moreover, silencing *tir-1* prevented the nuclear translocation of SKN-1 induced by PA14 infection, but did not affect its baseline expression levels. Altogether, these results suggest that the TIR-1/PMK-1 pathway is necessary to attain activation of SKN-1 by PA14 exposure.

Involvement of SKN-1 in immunosenescence

Immune function declines with age, leading to compromised immune responses to infections in the elderly. As SKN-1 is required for both longevity and pathogen resistance, I asked if chronological aging affected SKN-1-dependent target gene expression in nematodes exposed to pathogenic stress. To this end the promoter induction of *gcs-1* by PA14 was monitored in L3 stage larvae, 4-day and in 9-day old adult worms, respectively. A massive age-dependent decrease was observed in the expression of *Pgcs-1*::GFP reporter after 24 h of PA14 infection ($p<0.0001$). To address the potential involvement of SKN-1-dependent gene expression in immunosenescence, we performed a bioinformatics analysis using previously published microarray databases. From the 379 genes exhibiting the most significant down-regulation during aging (>10 fold down-regulation at d15 *vs.* d6) 46 SKN-1-regulated genes were identified. Strikingly, SKN-1-regulated genes subject to PA14-dependent regulation were over-represented (65%) compared to those regulated by either oxidative stress (30%) or PMK-1 (28%), respectively. These results suggest that the age-dependent decline in SKN-1 target gene expression may contribute to immunosenescence in *C. elegans*.

To investigate, how SKN-1 activity is involved in immunosenescence, we examined the survival of 1, 4 and 9 day-old adult N2 and *skn-1(zu135)* mutant nematodes exposed to PA14. We observed that pathogen resistance in wild-type animals already declined at day 4. Consistent with a premature decline of self defense in

the absence of SKN-1 activity, 4d adult N2 worms showed similar survival on PA14 to 1d adult *skn-1(zu135)* animals (p=0.1429). Furthermore, we found that *skn-1(zu135)* mutant animals exhibited increased susceptibility to PA14, compared to N2 at all ages (p>0.0001) indicating that SKN-1 function is also required to survive infection beyond day 9. These results confirm a progressive age-dependent compromise in pathogen resistance and imply that a decline in SKN-1 function contributes to immunosenescence.

Reduced insulin/IGF signaling requires SKN-1 for enhanced pathogen resistance

Loss-of-function mutations in the insulin/IGF-1 receptor gene, *daf-2* enhance stress resistance and extend lifespan, and both processes require DAF-16 and SKN-1 activity. As reduced insulin/IGF signaling (IIS) increases pathogen resistance, I investigated the contribution of SKN-1 to pathogen resistance in *daf-2(e1370)* mutant animals. In accordance with previously published data, *daf-2(e1370)* mutants exhibited robustly increased pathogen resistance against PA14 (p<0.0001). However silencing *skn-1* by RNAi largely increased their susceptibility to PA14 (p<0.0001). These data suggest that SKN-1 is required for reduced IIS to bring about enhanced pathogen resistance against *P. aeruginosa*.

Oxidative preconditioning induces pathogen resistance in an SKN-1- and DAF-16-dependent manner

Exposure to mild stresses induces tolerance to a lethal challenge, cross-tolerance to other stresses and extends lifespan. To address the impact of oxidative preconditioning on pathogen resistance, nematodes were pretreated with 1 mM, 1.5 mM and 2 mM H_2O_2, and then exposed to PA14 infection. H_2O_2 preconditioning induced resistance against PA14 in a concentration-dependent manner (p=0.4253, p<0.0001, p<0.0001, respectively), reaching a 2-fold increase in survival by 2 mM H_2O_2, compared to untreated controls. Intriguingly, the same treatment on *skn-1(zu135)* mutant nematodes not only exhibited a decreased pathogen resistance (p<0.0001), but had a strongly suppressed reaction to H_2O_2 compared to wild type animals (p=0.0156 vs p<0.0001). We also found that the loss of function mutation of another major oxidative

stress response regulator, DAF-16 (*daf-16(mu86)*) caused poor response to H_2O_2 compared to wild type animals ($p=0.0304$ vs $p<0.0001$). Thus, oxidative preconditioning requires both SKN-1 and DAF-16 for enhanced pathogen resistance against *P. aeruginosa*.

Excessive activation of SKN-1 by *wdr-23(RNAi)* impairs pathogen resistance

Finally, I investigated whether increased activation of SKN-1 was able to promote pathogen resistance. Stabilization of SKN-1 by RNAi against *wdr-23* has been shown to induce constitutive SKN-1 activation, resistance to oxidative stress and longevity. Feeding worms with *wdr-23(RNAi)* indeed resulted in an unexpectedly robust increase in the expression of *Pgcs-1*::GFP and GST-4::GFP compared to the PA14-induced expression. *wdr-23(RNAi)*, compared to empty vector feeding greatly reduced pathogen resistance to PA14 ($p<0.0001$). *wdr-23(RNAi)* did not impair survival in a *skn-1(zu135)* mutant background ($p=0.1992$) excluding a SKN-1-independent impact of WDR-23 on pathogen resistance. Determination of oxidative tolerance revealed that *wdr-23(RNAi)* animals exhibited increased survival ($p<0.0001$, $p<0.0001$), whereas *skn-1(RNAi)* nematodes displayed decreased surviva ($p<0.0001$, $p<0.05$), compared to control worms, when exposed to 3 mM or 5 mM H_2O_2, respectively. Our results suggest that an excessive post-translational stabilization of SKN-1 induces oxidative stress resistance but impairs resistance to bacterial infection.

DISCUSSION

NRF2 is a master regulator of oxidative stress response. As oxidative stress occurs in many diseases, NRF2 became a novel drug target in the last decades. During my Ph.D. work I created a manually curated NRF2 interaction database harboring 108 NRF2-interacting proteins, an order of magnitude higher than deposited interactions in the current web resources. This database can serve as a valuable source for NRF2 researchers and help us understand the complex regulation of NRF2, which could be essential to develop more efficient drugs against civilization diseases such as cancer, neurodegenerative diseases and chronic obstructive pulmonary disease.

Investigation of GO functions of NRF2 interacting partners showed a complex role of NRF2 in various biological processes. The analysis identified five known functions of NRF2, and predicted further three, such as development, reproduction and immunity. Although the last three biological processes were not listed among the annotated NRF2 GO functions, there are published data about the involvement of NRF2 in these functions in other model organisms. In mammals, the role of NRF2 in immunity (modulation of inflammation) has already been described, that I also confirmed in the nematode, *Caenorhabditis elegans* in the second part of my Ph.D. work. The analysis of NRF2 interacting partners and functions demonstrated that NRF2 is involved in diverse processes, thus, the manipulation of NRF2 activity requires precise engineering in case of drug development.

In the second part of my Ph.D. work I investigated the role of NRF2/SKN-1 in *C. elegans* immunity. The nematode *C. elegans* is a versatile tool to study the ancient, NF-κB-independent mechanisms of innate immunity. The activation of SKN-1 in the intestinal cells upon immune response suggests a role for NRF2 in mammalian immune cells during anti-bacterial defense. In *C. elegans* SKN-1 is activated by the TIR-1/PMK-1 pathway upon immune response. Due to the differences in SKN-1 and NRF2 regulation, the activation of NRF2 via the SARM/p38 MAPK pathway requires further investigation.

Emerging data suggests a general age-dependent decline in the inducibility of stress response regulators, e.g.: HSF-1, DAF-16/FOXO. In accordance with this

phenomenon, SKN-1 less efficiently activates the expression of its target genes upon infection during aging. A proper stress response is fundamental to maintain the (protein) homeostasis of the immune cells upon anti-bacterial defense. Decline in the activity of other stress transcription factors and signaling proteins might also contribute to immunosenescence. Discovering the involved factors can lead to better understand and help delay immunosenescence. As the role of NRF2 in immunosenescence has not been revealed yet, my results in *C. elegans* can open a new direction in this field.

Exposure to mild oxidative stress induces tolerance to a lethal challenge, cross-tolerance to other stresses and extends lifespan. I demonstrated that mild oxidative preconditioning increases pathogen resistance against *P. aeruginosa*. The enhanced resistance required both SKN-1 and DAF-16 proteins, which suggests a cooperation between the two antioxidant transcription factors. The fact that longevity and enhanced pathogen resistance caused by reduced IIS also requires both proteins seem to confirm this hypothesis. Discovering the interaction between stress response transcription factors may provide valuable results for researchers in the aging and stress fields.

Excessive activation of SKN-1 increased resistance against oxidative stress while compromised pathogen resistance, which suggests that SKN-1 may act in these processes *via* different mechanisms, e.g. by activation of specific target gene profiles. If NRF2 acts similarly in these processes as SKN-1, drug development shall aim the required NRF2 target gene profile, not only the over-activation of the protein. Investigation of the NRF2 target gene profiles under different conditions may promote the determination of the proper drug targets.

In summary, my Ph.D. I demonstrated how combined use of bioinformatics and experimental approaches promote the elucidation of the diverse biological roles of the prime transcriptional regulator, NRF2.

LIST OF PUBLICATIONS

Publications directly related to the thesis:

Papp D., Csermely P., Soti C. (2012) A Role for SKN-1/Nrf in Pathogen Resistance and Immunosenescence in *Caenorhabditis elegans*. *PLoS Pathog* 8:(4) e1002673 (IF: 9.127)

Papp D., Lenti K., Modos D., Fazekas D., Dul Z., Turei D., Foldvari-Nagy L., Nussinov R., Csermely P., Korcsmaros T. (2012) The NRF2-related interactome and regulome contain multifunctional proteins and fine-tuned autoregulatory loops. *FEBS Lett*. 586(13):1795-802 (IF: 3.538, independent citations: 1)

Publications not directly related to the thesis:

Cabreiro F., Ackerman D., Doonan R., Araiz C., Back P., **Papp D.**, Braeckman B. P., Gems D. (2011) Increased life span from overexpression of superoxide dismutase in *Caenorhabditis elegans* is not caused by decreased oxidative damage. *Free Radic Biol Med*. 51(8):1575-82. (IF: 5.423, independent citations: 10)

Dancso B., Spiro Z., Arslan M. A., Nguyen M. T., **Papp D.**, Csermely P., Soti C. (2010) The heat shock connection of metabolic stress and dietary restriction. *Curr Pharm Biotechnol* 11: 139-145. (IF: 3.455, independent citations: 5)

Biro A., Rovo Z., **Papp D.**, Cervenak L., Varga L., Fust G., Thielens N. M., Arlaud G. J., Prohaszka Z. (2007) Studies on the interactions between C-reactive protein and complement proteins. *Immunology* 121: 40-50. (IF: 3.398, independent citations: 35)

Papp D., Prohaszka Z., Kocsis J., Fust G., Banhegyi D., Raynes D. A., Guerriero V. (2005) Development of a sensitive assay for the measurement of antibodies against heat shock protein binding protein 1 (HspBP1): increased levels of anti-HspBP1 IgG are prevalent in HIV infected subjects. *J Med Virol* 76: 464-469. (IF: 2.52, independent citations: 3)

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