CLOSING REPORT
Hsp90-dependent regulatory mechanisms in aging

1. SUMMARY OF MAJOR ACHIEVEMENTS

1. Demonstration of a spatiotemporal requirement for Hsp90 function in development and adulthood
2. Hsp90 promotes wildtype and daf-2 longevity via DAF-16-dependent and independent mechanisms
3. Hsp90 is required for DAF-16 A activity and DAF-16A-mediated longevity

(Somogyvári et al., 2018)

4. Evidence of a conserved Hsp90-client interaction: Hsp90 stabilizes the longevity proteins mammalian SIRT1 and its *C. elegans* ortholog SIR-2.1
5. Hsp90 inhibition induces SIRT1 ortholog destabilization and proteasomal degradation

(Nguyen et al., 2018)

6. Hsp90 promotes body fat accumulation during development
7. Hsp90 and SIR-2.1 are required for fasting-induced lipid mobilization
8. Hsp90 in muscle promotes lipid mobilization in a cell non-autonomous manner
9. Proteotoxic stresses in muscle and in gut inhibit lipid mobilization

(Somogyvári and Sőti, unpublished – to be continued in a future project)

10. Hsp90 is required for macroautophagy in *C. elegans* and in mammalian cells

(Somogyvári, Holczer, Kapuy and Sőti, unpublished – to be continued in a future project)

11. 17-allylamino-17-demethoxygeldanamycin is a pharmacological *C. elegans* Hsp90 inhibitor

(Somogyvári and Sőti, unpublished – to be continued in a future project).

12. Generation of the mammalian Hsp90 client network and identification of novel physiological (GO) functions

(Fekete, Veres, Csermely and Sőti, unpublished)

13. Hsp90 chaperones the mammalian but not the *C. elegans* orthologs of DAF-16/FOXO3a, SKN-1/Nrf2, LET-363/TOR and DAF-15/RapTOR. Evidence of lack of strong conservation of the Hsp90 clientele.

(Nguyen, Somogyvári and Sőti, unpublished).

14. Hsp90 knockdown is a danger signal: induces cellular stress defenses and behavioral avoidance

(Gecse, Gyurkó and Sőti, unpublished)

15. Implementation and development of behavioral and learning assays
16. Demonstration of the *C. elegans* RasGAPS involvement in multiple processes in chemosensation, learning and memory formation

(Gyurkó et al., 2015)

17. Establishment of an early life toxic stress paradigm
18. Early life toxic stress does not induce imprinted (life-long) avoidant behavior in adults
19. The first evidence for an imprinted cellular stress defense memory: early life stress-associated sensory cues induce stress-specific transcriptional stress responses in adult worms

(Gecse et al., 2019)

20. Establishment of a paradigm of toxic stress and showing a critical role of homeostasis of somatic cells in regulating behavioral avoidance.
21. Identification of an underlying molecular mechanism involving toxin-specific stress and detoxification mechanisms relying on DAF-16/FOXO, Hsp90 and SKN-1/Nrf
22. Establishment of a connection between animals' ability to implement adequate cytoprotection during stress and future behavioral decisions through the formation of specific associative memories.

(Hajdú et al., 2020) preprint

23. A comprehensive review on the emerging role of Hsp90 in aging and longevity

(Somogyvári et al, manuscript in preparation)

Collaborations:

24. The identification of protein kinase D isoforms 1-3 as Hsp90 clients (Varga et al, manuscript in preparation)
25. High fat diet-induced metabolic stress does not affect Hsp90-dependent processes in rats (Bukosza et al., 2019)
26. Reviewing the stress-related roles of resveratrol (Grinan-Ferré et al, submitted to Aging Res Rev)

2. DETAILED REPORT

Aging is a the major risk factor for leading causes of death, including obesity, diabetes, neurodegeneration and cancer. Heat shock protein 90 (Hsp90) is a conserved molecular chaperone that regulates the heat shock response and stabilizes several hundred signaling 'client' proteins mainly involved in cell proliferation (Taipale et al., 2010) - for the sake of simplicity, only references of high importance are cited). In this project, investigated the role of Hsp90 in aging and stress-related processes, using the versatile model organism *Caenorhabditis elegans*.

2.1. Spatiotemporal role of Hsp90 for pleiotropic processes in *C. elegans*

Loss-of-function mutations of Hsp90 in nematodes, just as in mammals, are lethal. Nevertheless, as the few report on Hsp90 in the literature used mainly mutants, we spent more than half-a-year with the characterization of different Hsp90 mutants and showed that they do not exhibit the signs of reduced Hsp90 function. Moreover, we could not separate the effect of Hsp90 during and after development, which is key to have a stronger focus on aging. Hence, we reduced Hsp90 capacity in worms using two independent RNAi constructs, yielding identical results and systematically investigated all phenotypic consequences. Consistently with its critical role in cell proliferation in mammalian cells, we confirmed that Hsp90 was indispensable for oogenesis and embryogenesis, RNAi knockdown resulting in sterility and embryonic lethality in the F1 generation. Hsp90's role in development was confirmed and extended with the finding that Hsp90 is required to bypass dauer formation in neurons. Dauer larva is a stress-resistant persistent larval form which is induced in response to stresses, such as starvation and heat. Interestingly, the pathways responsible for dauer during development, such as the insulin-like singaling pathway (ILS), regulate longevity in adulthood. Thus, neuronal Hsp90 appears to buffer against the formation of a stress-induced alternative developmental pathway. This finding related to the role of nervous system in stress will gain importance later in the report. We also confirmed other phenotypes, such as a role in vulval development, reduced motility and the induction of the heat shock response (Somogyvári et al., 2018). These findings are consistent with numerous interactions in various tissues exhibited by Hsp90 throughout the worm life and represent the first systematic study on Hsp90 in *C. elegans*.

2.2. Hsp90 is required for normal and extended lifespan conferred by reduced ILS

To dissect the role of Hsp90 on longevity exerted during development and adulthood, respectively, we treated worms by hsp90 RNAi from the L1 larval stage and at the end of development. hsp90 RNAi in somatic cells shortened normal lifespan comparable from hatching throughout life and in adult life. Hsp90 reduction from L1 results in reduced fertility and and induces thermotolerance. These findings together show that Hsp90 is required for lifespan during adulthood, and its effect is independent on fertility. Moreover, its effect is not related to the induction of heat shock response and thermotolerance suggesting a different mechanism.

The insulin-signaling pathway is a major longevity determinant of mammalian lifespan and is dysregulated in various age-related diseases (Kenyon, 2010). We also studied the effect of Hsp90 on long-lived worms with reduced function of the daf-2 insulin-like receptor (Murphy and Hu, 2013). Hsp90 knockdown from hatching in daf-2 mutants and daf-2 RNAi fed worms resulted in similar phenotypes as in wildtype. Importantly, it significantly decreased their lifespan. Interestingly, RNAi feeding form adulthood showed a tendency to extend lifespan in daf-2 mutants, suggesting that the combined effect of Hsp90 knockdown together with the mild metabolic stress induced by lowered ILS induction of HSF-1 or a similar longevity mechanism. However, Hsp90 might be involved in pleiotropic pro- and anti-longevity processes, which are reorganized in daf-2 mutants.

2.3. Hsp90 is required for DAF-16A activity and DAF-16A mediated longevity

The DAF-16/FOXO transcription factor is a master regulator of oxidative and metabolic stress responses and is a major downstream effector of daf-2/ILS mediated longevity. Moreover, FOXO is the first gene, of which polymorphisms are linked to human longevity (Martins et al., 2016). We found that in both wildtype and daf-2 mutants, Hsp90's effect contained a daf-16-dependent, and daf-16-independent component. Interestingly, the extension of daf-2 lifespan by hsp90 RNAi was also daf-16 dependent, showing the involvement of a different hsp90-dependent pathway.

Next, we investigated the effect of Hsp90 on the activation of DAF-16. DAF-16 activity is regulated by its nuclear translocation in both by ILS and by stresses, such as heat shock. We observed that Hsp90 knockdown inhibited the nuclear translocation of DAF-16::GFP in response to daf-2 RNAi and heat shock. Just as in mammalian FOXO, *C. elegans* DAF-16 has several isoforms, out of which the A and the D/F have been reported to regulate lifespan (Chen et al., 2015; Kwon et al., 2010). We found that Hsp90 is required for the nuclear translocation of DAF-16A, but not DAF-16D/F. Likewise, hsp90 RNAi selectively inhibited the transcription of DAF-16A-specific target mRNA-s, but not those of DAF-16D/F, both in wildtype and in transgenic single DAF-16

isoform expressing strains. Finally, we demonstrated that in the single isoform expressing transgenic strains Hsp90 was required specifically for the longevity promoting effect of DAF-16A. A recent study showed that it is DAF-16A that plays a major role in longevity, whereas DAF-16D/F appears to be a backup. The combined results of that and our study suggest that Hsp90 capacity regulates ILS_mediated longevity via DAF-16A, but the Hsp90 independent DAF-16D/F may provide a backup in response to proteostasis collapse. Within a year after our study, two other reports citing our paper identified Hsp90 as a therapeutically relevant and druggable longevity regulator, highlighting the importance of these findings (Fuentealba et al., 2019; Janssens et al., 2019; Somogyvári et al., 2018).

We also observed that Hsp90 does not stabilize DAF-16A, excluding a chaperone-client interaction. Interestingly, we found that the mammalian ortholog FOXO3a is an Hsp90 client showing that the Hsp90 chaperone-client interaction is not entirely conserved between worms and mammals (Nguyen, Somogyvári and Sőti, unpublished). In parallel we have found that Hsp90 regulates DAF-16A activation upstream of its nuclear transport, probably by acting in the ILS pathway. Taken together, Hsp90, although by various molecular mechanisms, regulates the major longevity ILS/FOXO pathway activity during evolution.

We note that we experienced a long challenging time with the lifespan experiments, daf-2(e1370) strains, the daf-16 RNAi construct and the DAF-16D/F::GFP strain, which significantly retarded our publication and also the project for over a year.

2.4. The mammalian and worm SIRT1 orthologs are evolutionarily conserved Hsp90 clients

The SIRT1 sirtuin deacetylase orthologs are prominent regulator of lifespan and mediator of dietary restriction induced metabolic phenotypes from yeast to mammals (Imai and Guarente, 2014). In previous years we discovered that Hsp90 regulates adipogenesis (Nguyen et al., 2013), where SIRT1 also plays a role. Hence, we set out to study the interaction between mammalian and nematode SIRT1 and Hsp90 orthologs. We found that Hsp90 inhibition disrupts mammalian SIRT1 and the *C. elegans* ortholog SIR-2.1 stability and induces their proteasomal degradation. We also showed that Hsp90 forms a physical complex with SIRT1 that is disrupted by Hsp90 inhibition, which leads to the destabilization.

Our further experiments, in contrast to previous reports, showed insignificant effects of SIR-2.1 overexpression in the alternative ER UPR gene abu-11 expression (Viswanathan et al., 2005) and the mitochondrial UPR hsp-6 expression (Mouchiroud et al., 2013). Moreover, confirming our previous study (Burnett et al., 2011) we could not observe a lifespan extension by SIR-2.1 overexpression. Thus, as these processes were SIR-2.1-independent, we could not assess the impact of Hsp90 on SIR-2.1 physiological activity, which again casts doubt on the effect of *C. elegans* SIR-2.1 in these stress and longevity pathways (Somogyvári and Sőti unpublished). Although we observed that SIR-2.1 increased thermotolerance, the fact that Hsp90 also interfered with thermotolerance prevented us to test the interaction in this. We also note that this part of the project was again very time and labor consuming and did not result relevant data to be published (although we consider to publish it in a new journal Micropublication which publishes relevant negative findings, as well, to inform the community and save others' resources). Importantly, this is a first study that reveals an evolutionary conservation of an Hsp90-client interaction that exists in both roundworms and mammals (Nguyen et al., 2018).

2.5. Hsp90 is a regulator of lipid metabolism

SIR-2.1 has been reported to be required for lipid mobilization in worms (Walker et al., 2010). We investigated the role of SIR-2.1 in lipid mobilization induced by fasting (dietary deprivation) in nematodes. We observed that sir-2.1 mutation and sir-2.1 RNAi inhibited lipid mobilization in response to fasting. sir-2.1 did not interfere with lipid content during development.

Next, we tested how Hsp90 affects adipogenesis and adipolysis by staing worms with lipid stain Oil Red O. Due to the difficulty of evaluation, this experiment took 3 months to set up. First, we found that hsp90 RNAi from hatching resulted in reduced lipid content, suggesting a regulatory role for Hsp90 in lipid accumulation in a sir-2.1-independent mechanism, which requires further investigation. hsp90 RNAi from both hatching and in adulthood similarly inhibited fasting induced lipid mobilization. We aimed to test the relationship of hsp90 and sir-2.1. However, contrary to the abovementioned report (Walker et al., 2010), SIR-2.1 overexpression (tested in two independent transgenes) did not augment adipolysis. Thus, from these experiments we were unable to clearly identify whether Hsp90 acts through SIR-2.1.

We were curious whether Hsp90 exerts a cell autonomous function in the gut, the major lipid storage and metabolic organ in the worm. Using a gut-specific RNAi sensitive strain we found that sir-2.1 RNAi inhibits, whereas hsp90 RNAi does not affect lipid content in response to fasting. Thus, sir-2.1 is required and is sufficient in the gut for lipid mobilization, but hsp90 inhibition in the gut is insufficient to induce a disturbance and/or Hsp90

appears to exert a cell non-autonomous function. As the RNAi does not penetrate neurons in wildtype, we speculate that it might be the muscle or the hypodermis. As daf-16 RNAi does not affect adipolysis, these results also indicate an effect that is not directly related to SIR.-2.1 or DAF-16.

Dietary deprivation induces lifespan extension in an hsf-1-dependent manner in nematodes (Steinkraus et al., 2008). As hsp90 inhibition induces hsf-1, we tested the hsf-1-dependence with no effect of mutation or RNAi knockdown. Thus, hsp90 functions independently of hsf-1. This is supported by the fact, that heat shock, an activator of hsf-1 inhibited fasting induced adipolysis. Experiments to identify the target will be pursued during a future project (Somogyvári, submitted proposal).

Obesity is a major risk factor for age-related metabolic and other diseases. Therefore, as part of a collaboration, we have tested whether high fat diet and the induced metabolic stress would affect Hsp90-dependent processes in rats. However, we have not found a significant alterations in Hsp90 mRNA, protein levels and Hsp90-dependent client proteins in this model (Bukosza et al., 2019).

2.6. Proteotoxic stress regulates Hsp90-dependent processes

Heat shock is an archetype of proteotoxic stress, which induces protein misfolding. Proteotoxic stresses overload Hsp90 capacity and result in the destabilization of clients and disruption of pathways, resulting in evolutionary drifts in Drosophila and the regulation of mammalian adipogenesis (Nguyen et al., 2013; Taipale et al., 2010). To more specifically investigate the effect of proteotoxicity, we used different unstable aggregation-prone protein constructs expressed in muscle or in intestine. We found that the muscle-specific Hsp90 client unc-54 mutant protein exhibited Hsp90-dependent phenotypes, such as decreased motility and induction of the heat shock response at the restrictive temperature. Importantly, both the unc-54 as well as a gut-specific promoter driven Q82 polyglutamine peptide inhibited fasting-induced lipid mobilization, suggesting that the protein homeostasis in both tissues regulates lipid metabolism. Moreover, hsp90 silencing did not further increase this effect in mutants, indicating they act in the same pathway. As a control, the non-proteotoxic stressor benzaldehyde that activates both DAF-16 and SKN-1 in the gut did not interfere with lipid mobilization. These findings connect two major age-related pathologies through Hsp90 and suggest that protein homeostasis disturbances in other tissues might lead to obesity via the inhibition of adipolysis (Somogyvári et al, work in progress). Further work will be done to identify the underlying molecular mechanism.

2.7. Hsp90 is required for macroautophagy in *C. elegans* and in mammalian cells

Autophagy is a major downstream modulator of the aging process in diverse model organisms (Rubinsztein et al., 2011). Therefore, we investigated how hsp90 silencing affects macroautophagy. To this end, we used a GFP/mCherry-tagged LGG-1 autophagy reporter strain. Consistently with the literature, we observed, that daf-2 knockdown activated autophagy. Surprisingly, hsp90(RNAi) also appeared to activate autophagy. When we analyzed the effect of fasting, we found that it did not activate autophagy. Thus, we re-analyzed our assay and it turned out that the misleading results were largely due to technical difficulties, especially with the fluorescence microscope. We implemented a different method for the quantification of autophagic reporter fluorescence. In these conditions we found that the induction of autophagy by daf-2 knockdown was very mild, whereas fasting generated a stronger signal. Thus, we focused on the effect of fasting. Importantly, we observed that hsp90 RNAi inhibited the induction of autophagy in response to fasting. In cell cultures in collaboration with Orsolya Kapuy's group we observed that Hsp90 inhibition indeed inhibits autophagy and Hsp90 is required for the stability of ULK-1, which confirms a previously published evidence (Joo et al., 2011). Therefore, we are following this direction and utilizing the combination of cell culture and nematode models and will test if UNC-51, the *C. elegans* ortholog of ULK-1 is an Hsp90 client. Likewise, we aim to test whether autophagy plays a role in fasting induced adipolysis in the course of a future project (Somogyvári, Holczer, Kapuy, Sőti, unpublished and Somogyvári, submitted proposal).

2.8. 17-AAG is a pharmacological *C. elegans* Hsp90 inhibitor

To find means to pharmacologically inhibit C. elegans Hsp90, we screened a number of compounds including cisplatin and novobiocin, known C-terminal Hsp90 inhibitors from our previous studies, (Söti et al., 2002). These compounds exhibited strong and aspecific toxicity and did not inhibit Hsp90. As a further attempt, we collaborated with the Blagg lab, using a number of Hsp90 inhibitors developed by them, including KU135 and KU174. However, we did not observe any signs of Hsp90 inhibition.

Geldanamycin was the first Hsp90 inhibitor identified in mammalian cells. *C. elegans* Hsp90 remains unaffected by geldanamycin, therefore we did not test its derivatives. However, others found that it is inhibited by a derivative, 17-allylamino-17-demethoxygeldamycin (17-AAG) (Fuentealba et al., 2019), currently in clinical trials

as an antitumor agent. Therefore, we systematically analyzed its effect on the Hsp90-dependent phenotypes and molecular processes and found reduced body size and infertility, compromised including DAF-16 activation, adipolysis and autophagy in a dose-dependent manner. Our findings independently confirm it as an Hsp90 inhibitor and shows the druggability of Hsp90 as an aging target (Somogyvári and Sőti, work in progress).

2.9. Building the mammalian Hsp90 client network and its aging-related functions

In contrast to yeast and mammals, there is almost no data on Hsp90-protein interactions in *C. elegans*. Therefore, in collaboration with Péter Csermely's LINK group we built a topological map of the human Hsp90 client protein network using the human SignaLink 2.0 signaling resource database and Cytoscape. Next, we performed a GeneOntology enrichment analysis on the list of human protein kinases ranked by their interaction score to the Hsp90 protein, using G-profiler. Besides two assigned GO processes (Axon guidance, Innate and adaptive immune response) we identified four new Hsp90-related physiological processes, which are not listed in the GO database: Immune system development, Extrinsic apoptotic pathway, Stress activated MAPK pathway activation, Inhibition of the insulin pathway. Especially reminiscent finding the "Inhibition of the insulin pathway", to which our daf-2 longevity studies may provide experimental evidence. Immunity is highly important for longevity, and there were reports on Hsp90 in pathogen resistance in worms, also via HSF-1 (Singh and Aballay, 2006).

2.10. Studying and disproval of general conservation of the Hsp90 clientele in *C. elegans*

We have found that SIRT1 (Nguyen et al 2019), FOXO3a and Nrf2 (Nguyen and Sőti, work in progress) and protein kinase D1-3 isoforms (Varga et al, manuscript in preparation) are Hsp90 clients in mammalian cells. Further, mTOR and Raptor has also been reported as an Hsp90 client (Delgoffe et al., 2009). However, we found that apart from SIR-2.1, hsp90 RNAi did not affect DAF-16 protein levels, SKN-1/Nrf protein and reporter expression, LET-363/TOR and DAF-15/Raptor proteins and target gene expression (Nguyen, Somogyvári and Sőti, work in progress). This is consistent with the fact that Hsp90 clients have no special consensus motifs, but a thermodynamically unstable conformation. Unfortunately, these negative findings, although the idea was promising, did not yield publications. As we could not establish a reliable *C. elegans* Hsp90 clientele with merely bioinfomatics tools based on the conservation, which would have been needed for simulations and predictions, we were unable to do simulations. Rather, we will identify the worm clientele in a future project using systems biology tools (Somogyvári, submitted grant proposal).

2.11. Hsp90 RNAi in *C. elegans* reveals a link between stress responses and behavior

While investigating the stress resistance of hsp90 RNAi fed worms, we observed that worms avoided the bacterial lawn of the hsp90 RNAi expressing strain in the absence of toxic agents. Indeed, Hsp90 knockdown in somatic cells was perceived as a toxic stimulus and associated with the odor of the bacteria harboring the RNAi construct (Gecse, Gyurkó and Sőti, unpublished). A pioneering study reported that compromise of vital cellular functions (such as translation, energy production) induce behavioral avoidance and immune responses and concluded that the organism monitors the core cellular processes and interprets their disruption as toxic or pathogen attack (Melo and Ruvkun, 2012). Our results show that Hsp90 function belongs to this core process set and its reduction is transduced into the neural system, interpreted as a danger signal and induces aversive behavior. Moreover, worms learn the odor and upon reencounter recall the stress-associated memory. These findings are intriguing, because stress responses form a cellular defense, whereas behavioral avoidance forms an organismal first line defense against stresses, but their connection is largely unknown. We decided to further investigate this highly relevant connection due to several reasons: *(i)* others and we showed that stress responses collapse during aging (Papp et al., 2012; Taylor and Dillin, 2011) *(ii)* early life stresses induce lifelong consequences via genomic and behavioral imprinting (Tucci et al., 2019; Wilson and Sullivan, 1994) *(iii)* the coordination between cytoprotective stress responses in each cell and the behavioral defense is unknown *(iv)* as outlined before, we had several technical difficulties during the project and although we could not plan it at the proposal, it appeared a very promising direction with relevance to Hsp90, stress responses and aging.

To this end, we have implemented and further developed a set of different assays to study neurobehavior and learning, we have confirmed the neural chemosensory and peripheral motility defects of hsp90 silenced nematodes and demonstrated the *C. elegans* Ras GTPase Activating Proteins (RasGAPS) involvement in multiple processes in chemosensation, learning and memory formation (Gyurkó et al., 2015).

2.12. A cellular defense memory imprinted by early life stress

Early in life, both the cells as well as the developing nervous system is very sensitive to environmental inputs. For instance, behavioral imprinting (such as the homing of salmons and preference of perinatally experienced food odors in humans (Wilson and Sullivan, 1994) creates a life-long memory which serves as the biological basis of secure, long lasting attachment to qualities essential for individual and/or species' survival. Besides, early life stresses (such as starvation) lead to various human age-related metabolic and cardiovascular diseases, and cognitive and emotional disturbances in rodents (Debiec and Sullivan, 2017). Whetther the memory of early life stresses may also be specifically imprinted is unexplored. In *C. elegans*, it has been shown that pathogen exposure in the critical perinatal period elicits aversive behavior in adults, providing the first evidence of an imprinted memory by stress (Jin et al., 2016). However, whether this imprinting is limited to immunity or is a more general response to stress was unknown.

We established an early life stress paradigm in worms and investigated how the disturbance of the core cellular processes, including Hsp90 in the critical period influences cellular and behavioral stress responses in larvae an in adults. We found that RNAi against Hsp90, the proteasome and energy metabolism induced both cellular stress reporters and behavioral aversion after the critical period, but these responses were not present in adults because of the inability of RNAi to inhibit the maternally inherited proteins (Gecse and Sőti, unpublished). Therefore, we employed various toxins. (Unfortunately, at the time of these experiments we were not aware of the efficiency of 17-AAG against Hsp90.) We found that exposure to antimycin A and paraquat in the OP50 bacterial food lawn induced both agent-specific cellular stress reporters and food aversion in the critical period. After growing up worms in other bacteria we exposed adults to OP50 (without toxins). To our surprise, aversive behavior to OP50 cues did not persist into adulthood. But we made a striking observation: OP50 sensory cues simulated the respective stress-specific cellular reporters. Likewise, the respective toxins induced a cellular (genomic) imprinting of increased stress resistance to the respective toxins. Unfortunately, my PhD student finished her doctoral period and we needed to publish this paper. This is a first evidence on an imprinted (life-long) transcriptional cellular stress response. Although in the absence of the neural mechanism we could not communicate it in a top journal. The already 499 accesses since its publication in December 2019 shows its relevance (Gecse et al., 2019), which requires further systematic work.

2.13. Toxic stress-specific cytoprotective responses regulate learned behavioral decisions in C. elegans

Among the toxins we employed food derived volatile odors, which are attractive at low but aversive in high concentration. We found that aversion was a result of toxicity. Hsp90 RNAi specifically decreased stress tolerance to benzaldehyde (BA) and is involved in the cytoprotective responses against BA toxicity. Importantly, these odors possess both the attractive chemosensory cue and the aversive toxic property, which offers an opportunity to study the connection of cellular stress responses and learned behavioral responses. BA induced widespread but specific cytoprotective stress and detoxification responses, including DAF-16 and SKN-1 nuclear translocation and target gene promoter activation. Upon re-exposure, worms do not avoid the food contaminated with toxic BA, but the silencing of Hsp90, DAF-16 and SKN-1 in somatic cells prevents the development of behavioral tolerance. The upregulation of these responses confer which confer behavioral cross-tolerance to methyl-salicylate, which exhibits a similar chemical structure. A structurally unrelated food volatile diacetyl (DA) also induces aversion. But a preconditioning exposure augments diacetyl aversion and does not induce apparent molecular defenses. These findings show that the disturbance of cellular homeostasis by toxins are transduced into neurons and stimulate aversive behavior, but the induction of cytoprotective stress responses suppress this response.

We also studied learned responses by exposing worms to low, naturally attractive concentration of the odors after toxic preconditioning. We found that worms avoid dilute DA, but not BA, showing that associative learning depends on the efficiency of cytoprotective stress responses. Moreover, after reinforcing these experiences the retrieval of memories leads to avoidance of food contaminated by DA and flexible behavioral decision to avoid BA only if there is an alternative, food-indicative odor. These findings *(i)* reveal a critical role of homeostasis of somatic cells in regulating behavioral avoidance *(ii)* provide an underlying molecular mechanism by the identification of toxin-specific cytoprotective responses involving conserved stress and detoxification regulators, such as Hsp90 *(iii)* and establish a connection between animals' ability to implement adequate cytoprotection during stress and future behavioral decisions through the formation of specific associative memories ((Hajdú et al., 2020) preprint, submitted).

2.14. Reviews

Resveratrol is a plant polyphenol phytoalexin with anti-aging properties which pleiotropic targets including SIRT1 and HSF1 (Putics et al., 2008). We (Somogyvári and Sőti) summarized its role in protein homeostasis and

the stress responses and co-authored a review with Christian Grinan-Ferré and Merce Pallas: "The pleiotropic neuroprotective effects of resveratrol: from antioxidant to epigenetic therapy" which has been submitted to Aging Research Reviews, the most prominent review journal in aging.

We are preparing a comprehensive review with Péter Csermely's group on "Hsp90: an emerging role in aging and longevity" to be submitted to Ageing Research Reviews.

3. CLOSING NOTE

Our publication output, although reaches the number of papers, do not reach the quality we aimed. The less elaborated output was due to several technical and personnel difficulties, such as the lack of colleagues experienced in *C. elegans* biology. Yet, we believe that the studies on the role of Hsp90 in longevity and DAF-16 and on Early life stress induced imprinted cellular stress responses are both significant discoveries with impact on the fields. Moreover, there are a number of highly important papers to be published in more prestigious journals, such as (Hajdú et al., 2020) in BMC Biology and the review on Hsp90 to Ageing Res Rev and other studies (Hsp90 in fat metabolism and in autophagy) resulting in potentially relevant publications and opening new research directions. During my 2009-2011 NNF-78794 grant two papers appeared, but after the closure within a year we published four papers (in PLoS Pathogens, Antiox Redox Signal, FASEB J and a collaborative study in Nature). I anticipate a similar outcome this year thanks to the support of the present OTKA grant.

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