Rescue strategies in Drosophila models of neurodegenerative diseases

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Chapter 5

Summarizing discussion and
future perspectives

High birth rates in the beginning and middle of the twentieth century together with the recent prominent advances in medical care are now resulting in an exceptional history trend of rising proportions of older people in the total population. From 1950 until 2013, the number of elderly quadrupled, and the current aging population is predicted to triple reaching over two billion people by the year 2050. Significant aging has tremendous consequences not only in economical and political spheres but also in the social processes and well-being of individuals. Prolonging of life corresponds with the higher prevalence of age-related disorders, a large group of which is composed of neurodegenerative diseases[1]. To date, there is no efficient medication for these diseases, and the treatment is mostly symptomatic. Therefore, a crucial challenge in the medical sector that our society confronts with nowadays is to increase the quality of life of patients suffering from neurodegeneration, if not by curing the diseases, then by postponing pathological processes. A rapidly aging population and the problems associated with it necessitate an urge to unravel the mechanisms underlying degenerative processes and to search for modifiers of these processes.

In this thesis, we present examples of two strategies of possible modulations of phenotypes associated with neurodegeneration. We have focused on two complex disorders: spinocerebellar ataxia type 3 (SCA3) and pantothenate kinase associated neurodegeneration (PKAN). The disease phenotype modifications are discussed in detail, and future research perspectives are suggested.

General treatment strategy: Treating secondary pathological processes in SCA3 by induction of the H_2S biosynthesis pathway

Our first study was focused on influencing secondary pathological processes, and to apply this strategy of treatment in a neurodegeneration model, for this we selected the *Drosophila* SCA3 model. We were able to suppress the eye phenotype of the flies bearing the toxic polyQ stretch by induction of the H_2S biosynthesis pathway, or transsulfuration pathway. Its upregulation in different pathological conditions had been demonstrated to be beneficial in processes such as oxidative stress[2-4], inflammation[5] and by protecting mitochondrial function and integrity[5, 6]. In our study, it appeared that CSE overexpression was capable of partially rescuing the eye phenotype of the *Drosophila* model for SCA3. Studying molecular effects of CSE overexpression in detail revealed that the rescue was associated with reduction of the SCA3 induced immune response and oxidative stress. On the contrary, no effect on protein aggregate formation was observed in these flies. These results are consistent with the effect of supplementation of sodium thiosulfate to the food. Sodium thiosulfate is a donor of H_2S and an FDA-approved medicine used for treatment of other disorders[7, 8]. Finally, our results provided evidence that the positive impact of overexpression of CSE was executed through the partial recovery of the decrease in protein persulfidation levels that occurs in the SCA3 background. Altogether, the results of this study suggest that activation of the transsulfuration pathway is a suppressor of the SCA3 phenotype in *Drosophila* and this function is executed by protein persulfidation on the level of secondary pathological processes, and not by influencing the more primary causes of the disease. This conclusion supports the hypothesis that manipulations of the transsulfuration pathway in SCA3 and hypothetically other polyQ disorders can be referred to as a general treatment strategy.

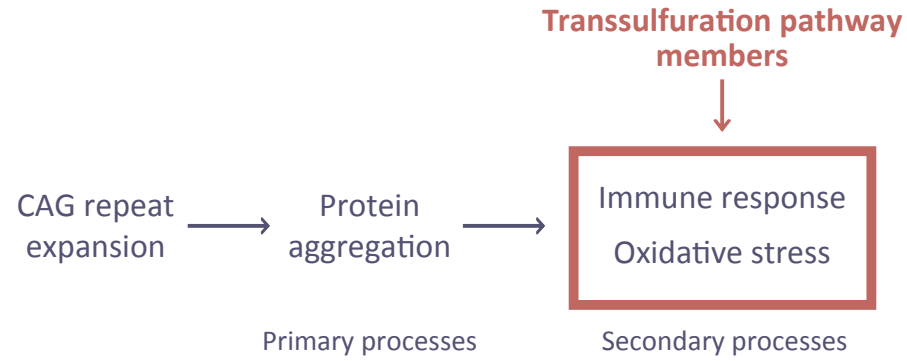

Figure 1. Schematic presentation of effects of transsulfuration pathway induction in the SCA3 *Drosophila* model
The cause of SCA3, the CAG repeat expansion, triggers protein aggregation, which is a primary pathological process. Next, secondary pathological processes, such as immune response and oxidative stress get involved, and this step can be modulated by induction of the transsulfuration pathway.

Specific treatment strategy: Modifying causal processes of the disease by replenishing CoA levels that are decreased in the *Drosophila* model of PKAN

In this chapter, we studied the specific rescue approach on *in vitro* and *in vivo* PKAN models by direct exogenous supplementation of the deficient compound, CoA, to cells or organisms via the medium and the food. Our research revealed a yet unknown source of CoA for CoA-deficient cells that employed uptake of exogenously supplemented CoA using only two of the classic enzymes (PPAT and DPCK) of the *de novo* CoA biosynthesis pathway. Supplemented CoA was extracellularly converted into 4'-phosphopantetheine, which translocated into the cells via passive transport and was then enzymatically converted to CoA. This novel finding in the field of fundamental cell biology can be potentially applied in therapy of CoA deficiency-related human diseases. In addition, the finding that bacteria can not obtain 4'-phosphopantetheine from their environment[9] and eukaryotic cells are able to take up 4'-phosphopantetheine[Chapter 3 of this thesis] reveals an interesting prospect of CoA-targeted treatment of microbial infectious diseases. Bacteria will die when a combination of CoA synthesis inhibitors and 4'-phosphopantetheine is added to their environment, whereas eukaryotic cells will survive.

In search for a specific treatment strategy unrelated to the cause of the disease: Generating a *Drosophila* PKAN model for large-scale studies with modifiable phenotypes

In our third study, we established a new *Drosophila* wing model of CoA impairment that can be used for large-scale screens. Previously it was impossible to perform such studies on existing PKAN models due to low numbers of eclosing flies and their poor viability. Another advantage of our wing model is the fact that it exhibits three distinctive phenotypes that are susceptible for modulation independently of one another. We validated our model by partial chemical rescue as well as by a small genetic interaction

study. This PKAN model can further be used not only to define the underlying pathological processes of the disease in a separate organ but also in a search for suppressors as potential treatment candidates or enhancers to see whether mutations in the latter are also implemented in the faster and/or more severe disease progression as observed in a subset of patients. So far, there is no explanation why some patients show a steeper decline of neurological functions compared to other patients with the same mutations.

Moreover, we used our model to test whether a general treatment strategy is applicable in our model. In order to do so, we measured the effects of the H_2S biosynthesis pathway, which is unrelated to the CoA biosynthesis pathway and had previously been shown to modify neurodegeneration[10-12, Chapter 2 of this thesis]. It appeared that CSE overexpression partially rescued one of the phenotypes associated with PKAN impairment, whereas CSE knockdown enhanced it. Such association between the dPKAN/Fbl knockdown-induced tissue degeneration and other pathways not directly involved in the CoA biosynthesis, suggests existence of other PKAN modifying genes and compounds independent of the primary cause of the disease. Using this model, we demonstrated that at least two of the phenotypes of the model could be modified: induction of H_2S biosynthesis partially rescued the held-out wing phenotype and supplementation of pantethine to the fly food rescued the smaller wing phenotype. These results prove that the phenotypes can be modified independently of one another.

Thus, our *Drosophila* wing model of PKAN can serve as a tool to identify novel candidates for a specific treatment strategy that are not associated with replenishing decreased CoA levels. These findings indicate a new point of view on possible therapeutic strategies of PKAN disease.

Research significance

Although the studies discussed in this thesis may eventually offer a medicine against the neurodegenerative disease PKAN, they do not provide a solution for neurodegeneration in general. However, we have investigated various treatment strategies via boosting the transsulfuration pathway that could potentially improve the state of neurodegeneration. In the present situation, when the numbers of elderly and, hence, neurodegeneration in patients is increasing, it would already be a great achievement to at least postpone pathological processes and broaden the spectrum of treatment possibilities if not cure the disease. As a result, this would lead to an improvement of life quality among aging population and decrease financial burdens caused by age-related diseases.

Moreover, the tactics of the study design described in this thesis illustrates the possibility to choose a general or specific approach of disease phenotype modulation in particular examples of two disorders, PKAN and SCA3. These strategies can be further applied for a broader range of diseases under investigation.

Finally, our findings reveal completely novel chemical and biological mechanisms. In the PKAN study, we demonstrate that 4'-phosphopantetheine directly crosses the cellular membrane and is able to replenish the intracellular levels of CoA. This finding hypothetically proposes a new replacement therapy for PKAN patients. Earlier, another precursor of CoA, pantethine, had been proven to rescue the PKAN *Drosophila* model[13]. However, in serum, pantetheine appeared to be rapidly converted into vitamin B5 and cysteamine by pantetheinases making it an inefficient therapeutic candidate[14-16]. The finding of 4'-phosphopantetheine being taken up by the cells and exhibiting rescue effects in the CoA impairment background offers a new therapeutic prospective on this molecule in PKAN. Also, our study proves the fact that, even after many decades of CoA research, there is a major lack of knowledge of its metabolism in organisms.

The study of effects of hydrogen sulfide biosynthesis pathway in SCA3 discloses not only the positive action of the gas in neurodegeneration but also the fact that protein persulfidation levels are affected in the disease and can be modulated.

In the study of our newly generated PKAN wing model, a pathway unrelated to the PKAN origin, H_2S biosynthesis pathway, is capable of modulating the disease phenotype. All these discoveries give a new prospective on the disease pathology and treatment.

FUTURE PERSPECTIVES

Hydrogen sulfide and its application in neurodegenerative diseases

Our data indicate a general rescue potential of the transsulfuration pathway in such pathological processes as, for example, oxidative stress response and inflammation[5, 17]. As discussed earlier, these processes are common for nearly all neurodegenerative disorders[18-20]. This implies that hydrogen sulfide could be potentially efficient in reducing and/or postponing secondary pathological processes taking place in various neurodegenerative diseases. Further studies in other neurodegenerative models of different origins need to be performed to confirm this hypothesis.

In our experiments, induction of hydrogen sulfide synthesis was achieved by CSE overexpression as increased protein persulfidation of CSE-overexpressing samples implies. Besides CSE, there are other enzymes implicated in the biosynthesis of H_2S. Cystathionine beta-synthase (CBS) catalyzes the first step in the transsulfuration pathway[21] and its overexpression enhances longevity upon dietary restriction[22]. Conceivably, CBS overexpression would play a modifying role in neurodegeneration as well as CSE does, and, hence, co-overexpression of CSE and CBS could be tested in various neurodegenerative models for a more distinct beneficial effect by a stronger induction of H_2S production.

We have demonstrated that thiosulfate, as a member of the transsulfuration pathway and a precursor of H_2S[23], exhibits protective functions similar to those of CSE overexpression in the SCA3 model. The choice of sodium thiosulfate (STS) as an H_2S donor was dictated by the fact that STS is a clinically approved compound[24]. Potentially this means that our discovery of the positive effects of the transsulfuration pathway could be relatively easy translated into the medical treatment of SCA3. However, other slow-releasing H_2S donors with a higher therapeutic potential have recently been developed as well[25-27]. Replacing supplementation of STS in the SCA3 model with supplementation of such a slow-releasing

hydrogen sulfide donor could enhance the rescue capacities of the compound-triggered induction of the transsulfuration pathway.

It has been recently proposed that hydrogen sulfide exerts its effects via protein persulfidation[28-30] and that protein persulfidation acts as a signaling pathway[31, 32]. Paul and Snyder propose that the role of protein persulfidation is even comparable in its prominence to the functions of phosphorylation[33]. However, besides the few studies on this topic, most assumptions remain speculative. Therefore, more extensive research of protein persulfidation as a signaling pathway in various molecular processes and tissues needs to be carried out. For example, it has been established that H_2S producing enzymes are expressed, among other organs, also in the brain[34-36]. Studies focused on the effects of hydrogen sulfide in neurodegenerative diseases discovered its altered levels in Parkinson's and Huntington diseases[12, 37]. Furthermore, a link between protein persulfidation and neuroprotective effects of parkin has been proposed[38], and the role of hydrogen sulfide and protein persulfidation in the brain has been acknowledged[39-41]. Nonetheless, the role of protein persulfidation as a signaling pathway in the physiological and pathological brain has been studied extremely poorly. As described earlier, our study of protein persulfidation levels in the SCA3 *Drosophila* model reveals decreased levels in the pathology. Thus, it is essential to investigate whether this phenomenon is a general event for all the neurodegenerative diseases or it is associated solely with SCA3. In case protein persulfidation is overall decreased in neurodegeneration, the next step would be to uncover specific proteins associated with the pathology and study how common they are for all the disorders.

As regards spinocerebellar ataxia type 3, further research should focus on detecting proteins, which are less sulfhydrated in the disease model and which protein persulfidation levels are restored by CSE overexpression. This can be done using the tag-switch assay with fluorescence labeling, with the following extraction of the affected proteins for mass spectrometry analysis. The obtained information might propose a mechanism of protective effects of the transsulfuration pathway. As a follow-up of such a study, one could question whether protein persulfidation could serve as a disease biomarker. It has been previously shown that nitrosylation of a serum alpha-synuclein could be a potent marker for Parkinson's disease[42]. Phosphorylation of different proteins can be used as a marker of various brain disorders[43-45]. In a similar manner, protein persulfidation could potentially be employed for diagnostic purposes in neurodegenerative diseases.

Considering association between hydrogen sulfide and pathological processes in the brain, it is important to pay particular attention to memory and learning. To our knowledge, only few studies have demonstrated positive effects of hydrogen sulfide on memory and learning in brain disease models[46, 47]. In the meantime, hydrogen sulfide might be a potent candidate for memory and learning modulation, and its function in these processes might be exerted via protein persulfidation signaling.

The role of CoA in the organism in general and in PKAN in particular

Our study of the alternative mechanism of replenishing intracellular levels of CoA proved that the commonly accepted theory about intracellular biosynthesis as the only source of CoA in the cell was

invalid. This means that even now, a century after the discovery of CoA, there is still a major lack of knowledge about CoA metabolism and the processes CoA plays role in.

One of the questions that remained unanswered is whether 4'-phosphopantetheine transport through the cell membrane is a physiological process or it was triggered by artificially high levels of extracellular CoA. In case it is one of the physiological functions, the recently proposed signaling role of CoA[48] might be exerted through 4'-phosphopantetheine. This hypothesis requires further investigation.

A great potential of our findings lies in the therapeutic possibilities that translocation of CoA inside the cell offers. Taking into consideration that pantethine, a precursor of CoA from the classic CoA biosynthesis pathway, is rapidly degraded[14, 15], both CoA and 4'-phosphopantetheine could be alternative candidates for treatment of pantothenate kinase associated neurodegeneration. Having said that, it is important to emphasize that CoA levels in PKAN patients have never been measured and it remains, therefore, a question whether it is the deficiency of CoA itself that causes the disease phenotype in humans. Thus, one of the main steps in unraveling the mysteries of PKAN and searching for the appropriate treatment should be a study of CoA levels in patients.

A novel *Drosophila* wing model of PKAN and its use in search for pathological processes and new treatment strategies of PKAN

Our third study of PKAN modifiers in the novel *Drosophila* wing model revealed that the transsulfuration pathways could modulate one of the disease phenotypes. Our results have proven our hypothesis about the possibility to modulate PKAN phenotype by modulating a pathway that had never been associated with CoA impairment before.

Another observation was the diversity of the phenotypes of the PKAN wing model and the fact that they can be modified independently of one another. Pantethine rescued only the wing size phenotype, whereas the held-out position was partially rescued by induction of the transsulfuration pathway. This suggests involvement of a broader range of genes and pathways affected downstream of CoA impairment and grants a possibility to discover other PKAN modifiers outside the CoA biosynthesis pathway.

Besides the approach of a candidate choice implemented in the current study, multiple ways of selecting candidates that modulate a phenotype can be applied in future studies in our wing model. One example is a genetic screen, in which one can determine the modulation of the phenotype by a selected group of genes or by performing a genome wide screen to identify unforeseen players[49]. Another widely used approach is a pharmacological screen (Agrawal *et al.*, 2005), possibly with an accent on the compounds that are already in use as FDA-approved medicines for other diseases.

The fact that the PKAN wing model exhibits three phenotypes, two of which can be modified, implies that there are several underlying processes downstream of CoA impairment causing these phenotypes. The held-out position of the wings is partially rescued by induction of the H_2S pathway, which is also know for its beneficial effects against oxidative stress[2-4]. This effect is also shown in our study of the SCA3 model[Chapter 2 of this thesis]. Increased oxidative stress is also associated with another *Drosophila* model of PKAN[13].

[50]. These two facts imply that it might be interesting to evaluate the oxidation processes taking place in the wing model of PKAN and distinguish the mechanisms, through which it might lead to the held-out position. The wing size has been associated with defects in such processes as proliferation[51] and cell death[52] and with several signaling pathways like Hippo and TOR pathways[53, 54]. Increased apoptosis has also been shown to contribute to the phenotype of another model of PKAN[50]. Therefore, it might be worth analyzing these processes and pathway in more detail and how they are association with the size phenotype of our developed PKAN wing model.

In case of proven association of any of those processes with CoA impairment in the wing model, there is a possibility that their dysregulation contributes to the PKAN phenotype in patients. Better understanding of the mechanism of phenotype development downstream of CoA impairment will provide an opportunity to target the dysfunctional pathways directly instead of treating the symptoms only. The latter is unfortunately the only available PKAN therapy at the moment.

In this thesis we have demonstrated three approaches in treatment of two models of neurodegenerative disorders: the general strategy focusing on influencing common secondary processes in pathology (**chapter 2**), the specific strategy aiming at curing the primary cause of the disease (**chapter 3**) and the specific strategy (**chapter 4**) that does not affect the obvious primary cause but is making use of disease specific modifiers not directly related to the "obvious" or known disease origin. As there are no efficient strategies to treat neurodegeneration yet, in further studies, it might be beneficial to combine the mentioned three strategies to achieve a higher efficiency in treating neurodegenerative disorders.

REFERENCES

1. United Nations, Department of Economic and Social Affairs, Population Division (2013).
World Population Ageing 2013.
2. Kimura, Y., Goto, Y. & Kimura, H. Hydrogen sulfide increases glutathione production and suppresses oxidative stress in mitochondria. *Antioxidants & redox signaling* **12**, 1-13 (2010).
3. Kimura, Y. & Kimura, H. Hydrogen sulfide protects neurons from oxidative stress. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **18**, 1165-1167 (2004).
4. Carballal, S. *et al*. Reactivity of hydrogen sulfide with peroxynitrite and other oxidants of biological interest. *Free Radic. Biol. Med.* **50**, 196-205 (2011).
5. Bos, E. M. *et al*. Hydrogen sulfide-induced hypometabolism prevents renal ischemia/reperfusion injury. *Journal of the American Society of Nephrology : JASN* **20**, 1901-1905 (2009).
6. Fu, M. *et al*. Hydrogen sulfide (H_2S) metabolism in mitochondria and its regulatory role in energy production. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 2943-2948 (2012).
7. Mishanina, T. V., Libiad, M. & Banerjee, R. Biogenesis of reactive sulfur species for signaling by hydrogen sulfide oxidation pathways. *Nat. Chem. Biol.* **11**, 457-464 (2015).
8. Kabil, O., Motl, N. & Banerjee, R. H_2S and its role in redox signaling. *Biochim. Biophys. Acta* **1844**, 1355-1366 (2014).
9. Jackowski, S. & Rock, C.O. Metabolism of 4'-phosphopantetheine in Escherichia coli. *Journal of bacteriology* **158**, 115-120 (1984).
10. Kida, K. *et al*. Inhaled hydrogen sulfide prevents neurodegeneration and movement disorder in a mouse model of Parkinson's disease. *Antioxidants & redox signaling* **15**, 343-352 (2011).
11. Xie, L. *et al*. Therapeutic effect of hydrogen sulfide-releasing L-Dopa derivative ACS84 on 6-OHDA-induced Parkinson's disease rat model. *PloS one* **8**, e60200 (2013).
12. Paul, B. D. *et al*. Cystathionine gamma-lyase deficiency mediates neurodegeneration in Huntington's disease. *Nature* (2014).
13. Rana, A. *et al*. Pantethine rescues a Drosophila model for pantothenate kinase-associated neurodegeneration. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 6988-6993 (2010).
14. Brunetti, D. *et al*. Pantethine treatment is effective in recovering the disease phenotype induced by ketogenic diet in a pantothenate kinase-associated neurodegeneration mouse model. **137**, 57-68 (2014).
15. Wittwer, C. T., Gahl, W. A., Butler, J. D., Zatz, M. & Thoene, J. G. Metabolism of pantethine in cystinosis. *J. Clin. Invest.* **76**, 1665-1672 (1985).
16. Kaskow, B. J., Michael Proffit, J., Blangero, J., Moses, E. K. & Abraham, L. J. Diverse biological activities of the vascular non-inflammatory molecules â€" The Vanin pantetheinases. *Biochem. Biophys. Res. Commun.* **417**, 653-658 (2012).
17. Tyagi, N. *et al*. H_2S protects against methionine-induced oxidative stress in brain endothelial cells. *Antioxidants & redox signaling* **11**, 25-33 (2009).
18. Evert, B. O. *et al*. Inflammatory genes are upregulated in expanded ataxin-3-expressing cell lines and spinocerebellar ataxia type 3 brains. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **21**, 5389-5396 (2001).
19. Block, M. L. & Hong, J. Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog. Neurobiol.* **76**, 77-98 (2005).
20. Lin, Michael T., Beal,M.Flint,. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature Nature* **443**, 787-795 (2006).
21. Evande, R., Ojha, S. & Banerjee, R. Visualization of PLP-bound intermediates in hemeless variants of human cystathionine beta-synthase: evidence that lysine 119 is a general base. *Arch. Biochem. Biophys.* **427**, 188-196 (2004).
22. Kabil, H., Kabil, O., Banerjee, R., Harshman, L. G. & Pletcher, S. D. Increased transsulfuration mediates longevity and dietary restriction in Drosophila. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 16831-16836 (2011).
23. Kolluru, G. K., Shen, X., Bir, S. C. & Kevil, C. G. Hydrogen sulfide chemical biology: pathophysiological roles and detection. *Nitric oxide : biology and chemistry / official journal of the Nitric Oxide Society* **35**, 5-20 (2013).
24. Farese, S. *et al*. Sodium thiosulfate pharmacokinetics in hemodialysis patients and healthy volunteers. *Clinical journal of the American Society of Nephrology : CJASN* **6**, 1447-1455 (2011).
25. Zhao, Y. *et al*. Controllable hydrogen sulfide donors and their activity against myocardial ischemia-reperfusion injury. *ACS chemical biology* **8**, 1283-1290 (2013).
26. Li, L. *et al*. Characterization of a novel, water-soluble hydrogen sulfide-releasing molecule (GYY4137): new insights into the biology of hydrogen sulfide. *Circulation* **117**, 2351-2360 (2008).
27. Li, L. *et al*. The complex effects of the slow-releasing hydrogen sulfide donor GYY4137 in a model of acute joint inflammation and in human cartilage cells. *J. Cell. Mol. Med.* **17**, 365-376 (2013).
28. Yang, G. *et al*. Hydrogen sulfide protects against cellular senescence via S-sulfhydration of Keap1 and activation of Nrf2. *Antioxid. Redox Signal.* **18**, 1906-1919 (2013).
29. Xie, Z. Z. *et al*. Sulfhydration of p66Shc at cysteine59 mediates the antioxidant effect of hydrogen sulfide. *Antioxid. Redox Signal.* **21**, 2531-2542 (2014).
30. Krishnan, N., Fu, C., Pappin, D. J. & Tonks, N. K. H_2S-Induced sulfhydration of the phosphatase PTP1B and its role in the endoplasmic reticulum stress response. *Sci. Signal.* **4**, ra86 (2011).
31. Gotor, C., Garcia, I., Crespo, J. L. & Romero, L. C. Sulfide as a signaling molecule in autophagy. *Autophagy* **9**, 609-

611 (2013).

32. Sen N, Paul BD, Gadalla MM, Mustafa AK, Sen T, Xu R, Kim S,Snyder SH,. Hydrogen sulfide-linked sulfhydration of NF-κB mediates its antiapoptotic actions. *Mol. Cell* **45**, 13-24 (2012).
33. Paul, B. D. & Snyder, S. H. H(2)S signalling through protein sulfhydration and beyond. *Nat. Rev. Mol. Cell Biol.* **13**, 499-507 (2012).
34. Diwakar, Latha,Ravindranath, Vijayalakshmi,. Inhibition of cystathionine-γ-lyase leads to loss of glutathione and aggravation of mitochondrial dysfunction mediated by excitatory amino acid in the CNS. *Neurochemistry International Neurochemistry International* **50**, 418-426 (2007).
35. Vitvitsky V, Thomas M, Ghorpade A, Gendelman HE,Banerjee R,. A functional transsulfuration pathway in the brain links to glutathione homeostasis. *The Journal of biological chemistry* **281**, 35785-93 (2006).
36. Linden, David R., Sha, Lei, Mazzone, Amelia, Stoltz, Gary J., Bernard, Cheryl E., Furne, Julie K., Levitt, Michael D., Farrugia, Gianrico, Szurszewski,Joseph H.,. Production of the gaseous signal molecule hydrogen sulfide in mouse tissues. *JNC Journal of Neurochemistry* **106**, 1577-1585 (2008).
37. Hu, L. *et al*. Neuroprotective effects of hydrogen sulfide on Parkinson's disease rat models. *Aging cell* **9**, 135-146 (2010).
38. Vandiver, M. S. *et al*. Sulfhydration mediates neuroprotective actions of parkin. *Nat. Commun.* **4**, 1626 (2013).
39. Abe, K. & Kimura, H. The possible role of hydrogen sulfide as an endogenous neuromodulator. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **16**, 1066-1071 (1996).
40. Morikawa T, Kajimura M, Nakamura T, Hishiki T, Nakanishi T, Yukutake Y, Nagahata Y, Ishikawa M, Hattori K, Takenouchi T, Takahashi T, Ishii I, Matsubara K, Kabe Y, Uchiyama S, Nagata E, Gadalla MM, Snyder SH,Suematsu M,. Hypoxic regulation of the cerebral microcirculation is mediated by a carbon monoxide-sensitive hydrogen sulfide pathway. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 1293-8 (2012).
41. Paul, B. D. & Snyder, S. H. Modes of physiologic H_2S signaling in the brain and peripheral tissues. *Antioxid. Redox Signal.* **22**, 411-423 (2015).
42. Fernandez, Emilio, Garcia-Moreno, Jose-Manuel, Martin de Pablos, Angel,Chacon, Jose,. Serum from Parkinson's disease patients presents excess of protein halogenation and nitrosylation, with anomalous nitrosylation of serum α-synuclein as potentially etiological factor. *Mol Neurodegeneration Molecular Neurodegeneration* **8**, 1-2 (2013).
43. Hampel, H., Blennow, K., Shaw, L.M., Hoessler, Y.C., Zetterberg, H., Trojanowski, J.Q.,Special Issue: Biomarkers of Alzheimers Disease and Dementia in Cerebrospinal Fluid and Blood,. Total and phosphorylated tau protein as biological markers of Alzheimer@?s disease. *Exp. Gerontol.* **45**, 30-40 (2010).
44. Blennow K, Vanmechelen E,Hampel H,. CSF total tau, Abeta42 and phosphorylated tau protein as biomarkers for Alzheimer's disease. *Mol. Neurobiol.* **24** (2001).
45. Crowe SL, Tsukerman S, Gale K, Jorgensen TJ,Kondratyev AD,. Phosphorylation of histone H2A.X as an early marker of neuronal endangerment following seizures in the adult rat brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **31**, 7648-56 (2011).
46. Xuan, A. *et al*. Hydrogen sulfide attenuates spatial memory impairment and hippocampal neuroinflammation in Î²-amyloid rat model of Alzheimer's disease. *Journal of neuroinflammation* **9**, 202 (2012).
47. Zhang, L., Jiang, C. & Liu, D. Hydrogen sulfide attenuates neuronal injury induced by vascular dementia via inhibiting apoptosis in rats. *Neurochem. Res.* **34**, 1984-1992 (2009).
48. Davaapil, H., Tsuchiya, Y. & Gout, I. Signalling functions of coenzyme A and its derivatives in mammalian cells. **42**, 1056-1062 (2014).
49. Bilen, J. & Bonini, N. M. Genome-wide screen for modifiers of ataxin-3 neurodegeneration in Drosophila. *PLoS genetics* **3**, 1950-1964 (2007).
50. Bosveld, F. *et al*. De novo CoA biosynthesis is required to maintain DNA integrity during development of the Drosophila nervous system. *Human molecular genetics* **17**, 2058-2069 (2008).
51. Schwank, G. & Basler, K. Regulation of organ growth by morphogen gradients. *Cold Spring Harb Perspect. Biol.* **2**, a001669 (2010).
52. Wu, C. *et al*. Pelle Modulates dFoxO-Mediated Cell Death in Drosophila. *PLoS Genet.* **11**, e1005589 (2015).
53. Parker, J. & Struhl, G. Scaling the Drosophila Wing: TOR-Dependent Target Gene Access by the Hippo Pathway Transducer Yorkie. *PLoS Biol.* **13**, e1002274 (2015).
54. Hariharan, I. K. Organ Size Control: Lessons from Drosophila. *Dev. Cell.* **34**, 255-265 (2015).