Life in the slow lane: a multi-omics approach to molecular adaptions in hibernating Syrian hamster liver
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Chapter 6

General Discussion
The aim of this thesis was to investigate the mechanisms underlying hibernation in Syrian hamsters at various levels, including epigenetic, RNA, metabolite, and protein, and to examine how these mechanisms affect the (in)activation of the immune system. The ultimate goal was to identify potential targets that could be used to reduce organ damage in a clinical setting. We found a metabolic switch from glucose metabolism to fatty acid oxidation during torpor resulting in differential metabolite levels in torpid and aroused hamster livers. A change in metabolism is known to affect epigenetic substrates and cofactors, which is in parallel to increased activity of HDACs and decreased H3K acetylation in the torpid hamster liver. Furthermore, we identified a role for the pentose phosphate pathway and MAPK pathway during torpor, which is suggested to regulate transcription and cell cycle during hibernation, respectively. Lastly, we found a delayed expression of secondary response genes that play a role in immune regulation, including the increase in expression of IL-6 in arousal early hamsters. The metabolic and epigenetic changes may underlie the differential immune system regulation in hibernation, which could aid in the identification of drug targets with relevance to, for instance, organ transplantation. As an initial step, we used RNA-Seq to map gene expression changes in hibernating Syrian hamster liver (Chapter 2). The total of 619 differentially expressed genes identified were further investigated using pathway analysis. This analysis uncovered differential regulation of commonly regulated pathways in hibernation such as metabolic pathways, but also identified lesser known pathways in hibernation: MAPK pathway and PP1 pathway. The MAPK and PP1 pathway are both involved in cell cycle regulation through activation of transcription factors (e.g. Myc) and downregulation of cell cycle inhibitors, respectively. Their involvement in cell cycle regulation in hibernation is substantiated by sub-pathways identified using pathway analyses: cell cycle arrest, cell division and cell proliferation. These results imply that MAPK and PP1 pathways regulate the halt and progression of the cell cycle throughout hibernation.

To further examine the downstream effects of differential gene expression on metabolism regulation, cross-omics was performed using data from proteomics, metabolomics and mitochondrial proteomics (Chapter 3). This study confirmed the downregulation of glycolysis and a shift towards lipolysis, as previously shown by Chazarin et al. (1,2). We also found liver ketogenesis and fatty acid beta-oxidation were consistently upregulated throughout torpor and arousal. Furthermore, proteins involved in transcription and translation were downregulated in torpor. Collectively, it is evident that metabolic processes and gene expression/translation are strongly regulated throughout hibernation. As epigenetic regulation is an important factor at the cross-section of gene expression and metabolism, we studied histone modifications and DNA methylation in the hibernating hamster (Chapter 4). In this study, we found 10 epigenetic enzymes in liver to be differentially expressed between summer control, torpor and arousal animals, of which the majority (7 differentially expressed genes (DEGs)) were histone modifying genes. We
measured histone acetylation levels of several lysines on histone 3 and 4 (H3 and H4) and activity of histone deacetylases (HDAC) and histone acetyltransferase (HAT) enzymes. An increase in HDAC activity was observed in torpor, which correlated with reduced levels of overall H3ac protein in torpor. Increased HDAC activity has also been reported in two studies conducted on brown adipose tissue and skeletal tissue in ground squirrels (3,4). More specifically, levels of acetylation on H4K12 and H4K16 were decreased in arousal, which is found to favor active transcription (5). The observed decrease in acetylation levels of H4K12 and H4K16 is consistent with the reversal of transcriptional depression that occurs in arousal (6). Thus, the observed epigenetic changes indicate a possible role in the regulation of transcriptional activation and repression during the hibernation cycle, which may provide insights into the mechanisms involved in this physiological process.

In addition to specific changes in metabolic pathways, the results in Chapter 2 also identified a large cluster of downregulated genes throughout hibernation that are part of the innate immune response. The rate of immune responses is directly influenced by the metabolic state. The availability of Acetyl-CoA is rate limiting for transcription factors such as NF-κB, p65 and acetyltransferases such as the P300 family that initiate epigenetic reprogramming of immune genes such as TNF-α, IL-1β, and IFN-γ. In Chapter 2 and 5, we showed that hamsters arousing from torpor demonstrated increased levels of the negative regulator of NF-κB: TNFAIP, and suppressed levels of mRNA expression of TNF-α and IL-6 in the liver after LPS injection. TNF-α is a primary response gene, while IL-6 is a secondary response gene. Delayed expression of secondary response genes is controlled by epigenetic regulation, as these genes have more condensed chromatin and require ATP-dependent nucleosome remodelling complexes for their transcription (7). In contrast, primary response genes such as TNF-α and IL-1β do not require nucleosome remodelling complexes for their transcription and hence epigenetic regulation is unlikely to explain the reduced TNF-α expression in arousal (Chapter 5). Altogether, Syrian hamsters display a delayed immune response to LPS, which could be explained by a condensation of chromatin by the reduced metabolic rate and the need for nucleosome remodeling in arousal. This result indicates that lowering a metabolic rate in clinical settings could aid in the reduction of inflammation.

**METABOLIC SWITCH TOWARDS PENTOSE PHOSPHATE PATHWAY AND LIPOLYSIS INSTEAD OF GLYCOLYSIS IN HIBERNATION**

During hibernation, several hibernating animals use gluconeogenesis during torpor, although the extent to which it is used varies among different species of hibernators. The Syrian hamster, European hamster (Cricetus cricetus), and hedgehog (Erinaceus europaeus) are among the hibernators that have been found to use gluconeogenesis during torpor (8–10). These animals heavily rely on stored lipids as a fuel source during
hibernation and use gluconeogenesis to regulate glucose levels in the blood and support energy metabolism while in torpor.

Maintaining glucose homeostasis during an overnight fasting period in mammals is typically reliant on stored glycogen (11). During a longer term fast or starvation, essentially all stored glycogen in the liver is depleted (after ~30 h of fasting), and de novo glucose synthesis or gluconeogenesis is responsible for the generation of glucose as a fuel for other tissues (12). Many hibernators switch from glucose to fatty acid metabolism when entering torpor (1,13), which was validated in RNA sequencing (RNA-Seq) of hamster liver both at an individual gene level and using GO analysis, and by assessing protein and metabolite levels (Chapter 2 and 3).

In line with previous findings (14,15), results described in this thesis confirm the downregulation of glycolysis during the hibernation season. The storage of glucose as glycogen is regulated in glycolysis through the downregulation of three regulatory subunits of protein phosphatase 1 (PPP1R3C, PPP1R10, and PPP1R3B). The PPP1R3B gene encodes the hepatic glycogen-targeting subunit of PP1, which is responsible for regulating glycogen synthesis in the liver. When glycogenolysis is activated, the inhibition of hepatic glycogen-associated protein phosphatase-1 (PP1-GL) by glycogen phosphorylase A prevents the dephosphorylation and activation of glycogen synthase, effectively suppressing glycogen synthesis (16). The regulation of PP1 and PP2 has been shown in several hibernators: decreased activity of PP1 and PP2a in torpid squirrels was reported in liver and brain respectively (17,18), and increased PP2c activity was found in skeletal muscle, brown adipose tissue, kidney, brain and liver (17,19). In Chapter 2, we showed downregulation of the three regulatory subunits of PP1 in torpor in the hamster. This downregulation may contribute to the shift from glucose to fatty acid metabolism as part of metabolic rewiring in hibernation.

During torpor, hibernators experience a reduction in metabolic rate and Tb, leading to a decrease in the production of ATP by oxidative phosphorylation. To compensate for this decrease in ATP production, hibernators rely on alternative energy sources, such as stored lipids and carbohydrates. In some hibernating animals, such as bears and ground squirrels, the pentose phosphate pathway (PPP) is believed to play an important role in providing the energy and substrates necessary for survival during periods of torpor. The PPP is a metabolic pathway that occurs in the cytoplasm of cells and plays a crucial role in the production of NADPH and ribose-5-phosphate, which are required for the synthesis of nucleotides and fatty acids, as well as for maintaining cellular redox balance. The pentose phosphate pathway is divided into two parts: the oxidative and non-oxidative PPP (Fig.2). The oxidative arm of the PPP is upregulated in hibernators during torpor, which leads to an increase in NADPH production (20). NADPH is used for lipid synthesis and antioxidant defense, which is crucial for protecting cells from oxidative damage during the re-warming process when torpor ends. The oxidative pentose phosphate
pathway is a major source of metabolic intermediates for biosynthetic processes such as nucleotide and amino acid biosynthesis, lipogenesis, and cellular antioxidant defense (Fig.2, (21)). Additionally, the non-oxidative arm of the PPP is believed to play a role in the regulation of glucose metabolism during hibernation, as it allows for the interconversion of glucose-6-phosphate and fructose-6-phosphate, which can be used for glycolysis and gluconeogenesis. The non-oxidative phase links the glycolytic pathway to the pentose phosphate pathway and it allows cells to break down ribose molecules, which can subsequently be used for nucleotide production (Fig.2). During torpor in hamsters, we confirmed that metabolites of the non-oxidative pentose phosphate pathway were upregulated, and were subsequently reversed upon arousal (sedoheptulose-7-phosphate, glyceraldehyde-3-phosphate, glucose-6-phosphate, D-ribulose-5-phosphate) (Chapter 3).

Ribulose-5-phosphate is a product of the oxidative phase and can be used in the non-oxidative phase to produce ATP in the TCA cycle and as a precursor for nucleotides. In torpor, consistent with a study in Richardson's ground squirrels (22), we found that the TCA cycle is inhibited at the level of the oxoglutarate dehydrogenase complex (OGDC), which converts oxoglutarate to succinyl-CoA (Chapter 2 and (22)), or at succinate dehydrogenase (SDH), the critical enzyme for the conversion of succinate to fumarate (23). The upregulation of non-oxidative PPP and increased levels of ribulose-5-phosphate in hibernation suggests a mechanism to replenish nucleotides lost during the torpor phase of hibernation in order to reactivate transcription during arousal.

As aforementioned, the pentose phosphate pathway is a major source of NADPH, which is required for the conversion of GSSG to GSH, an important antioxidant (24). The decreased activity of GSSG has previously been shown in ground squirrels (25). The NADPH system is also responsible for generating free radicals in immune cells by NADPH oxidase. These radicals are used to obliterate pathogens by an oxidative burst (which is found to be reduced in neutrophils during early arousal (Chapter 5)). Additionally, NADPH can be used in reductive biosynthesis of fatty acids. In Chapter 2 and 3 we showed a shift toward lipolysis in the hibernation season. More specifically, we found an upregulation of lipases in torpor and optimized lipid transport in torpor and arousal, whereas lipogenic processes are inhibited during torpor (Chapter 2). Furthermore, we found a switch from combustion of unsaturated fatty acids (torpor) to saturated fatty acids (arousal) in late torpor. This shift likely protects against lipid peroxidation, since unsaturated fatty acids are more prone to peroxidation by reactive oxygen species (26).

Lastly, increased ketogenesis is also an important glucose sparing mechanism, which aids in conservation of body protein, the major source of gluconeogenic precursors in fasting and hibernating mammals (27). The fat-derived ketone body in the ground squirrel (d-β-hydroxybutyrate (BHB)) was found upregulated in serum during torpor, in a reciprocal relationship with glucose (28). BHB is an essential energy source for the hibernating brain as it crosses the blood brain barrier and is metabolized in the TCA cycle (28). Proteins
involved in ketone body formation (ketogenesis) were upregulated during torpor, but not arousal (Chapter 2), suggesting that during torpor, ketogenesis is a source of energy for specific organs, e.g. the brain.

Altogether, we here show a decrease in glycolysis and oxidative phosphorylation in torpor of Syrian hamster liver, which is in line with previous findings in other hibernators (1,30). In order to ensure proper energy production throughout hibernation, the hamster activates lipolysis, ketogenesis and the catabolic pentose phosphate pathway instead of relying on glucose oxidation under summer conditions. In addition, inhibition of glycolytic pathways and activation of pentose phosphate pathway likely limits the production of reactive oxygen species, which has not been studied in hibernators before.
CHROMATIN CONDENSATION THROUGH HISTONE DEACETYLATION MAY REGULATE TRANSCRIPTION DURING TORPOR

Metabolism and epigenetics share interacting factors dynamically and reciprocally. Previously, it was described that transcriptional regulation is an important aspect of hibernation in ground squirrel, bats and bears (31), but how these changes are regulated remains to be investigated. In this thesis, using transcriptomics and proteomics, we confirmed a change in RNA expression throughout hibernation, by a reduction of proteins involved in RNA processing and transcription (Chapter 1). Moreover, in Chapter 3 we found that levels of metabolites involved in the formation of nucleotides (purine and pyrimidine metabolism) are downregulated in torpor and restored in arousing hamsters. Furthermore, the upregulation of the pentose phosphate pathway during torpor increases levels of ribose-5-phosphate which can be stored to replenish nucleotide levels during arousal.

Body temperature is often substantially decreased during hibernation. Cold temperatures (18°C) have been shown to compact chromatin (32), limiting binding of transcription factors. Given that hibernation is accompanied by a drop in Tb, it is conceivable chromatin condensation plays a role in the regulation of gene expression. Chromatin condensation has been documented in hibernating thirteen-lined ground squirrels because of increased DNA methyltransferase activity (33), but can also be brought about through histone deacetylation, which allows for compaction of DNA around histones and therefore restricts binding of RNA polymerases, reducing overall transcription (34).

In Chapter 4, we discussed numerous histone acetylation modifications. Proteomic data of Syrian hamster liver identified that H3 acetylation is affected during torpor, including decreased H3K18 and H3K23 acetylation in torpor and increased H3K27ac in arousal similar to ground squirrel (4,35,36). Furthermore, we discussed that the decrease in H3K18 and H3K23 acetylation indicate a potential increase in chromatin condensation and a state of transcriptional repression in the liver during hibernation, whereas the increase in H3K27ac in arousing hamsters may open chromatin, thus (re)activating transcription. Collectively, these data further support the hypothesis that modulation of histone acetylation is contributing to transcriptional repression in torpor vs arousal.

HDACs and HATs are enzymes that modulate chromatin structure through the (de)acetylation of histone lysines, resulting in the condensation or relaxation of chromatin structures. Previously in ground squirrels, it was found that HAT activity remained stable whereas HDAC activity increased in torpor (37). Consistently, we found an increase in HDAC activity in torpid hamsters, accompanied with a decrease in H3 acetylation, which both returned to summer euthermic levels in arousal. Furthermore, HAT activity remained consistent during hibernation of the hamster (Chapter 4).

Previously, it has been found that SIRT2 protein levels are increased in liver of the ground squirrel during arousal (38). Our RNA-Seq analysis indicated an upregulation of SIRT2 and SIRT7 expression during arousal of the hamster, which is consistent with decreased
acetylation of their targeted H4K16 residues (Chapter 2 and 4). SIRT2 and SIRT7 are predominantly located in the cytosol but can translocate to the nucleus, where they target H4K16. In turn, reduced H4K16ac is associated with a decrease in H3K9 acetylation (5). Consistent with this, we observed a decrease in H4K16ac and H3K9ac in our study (Chapter 4). Despite stable HAT activity throughout hibernation in ground squirrels, an increase in protein levels of the HAT PCAF was observed during torpor (55), a finding which was not observed in hibernating hamsters in our study (Chapter 4). Interestingly, we did observe decreased protein expression of KAT6A and KAT13D in torpor with normalization of KAT6A in arousal. KAT6A is an enzyme that acetylates lysine residues in histone H3 and H4. It is a main component of the MOZ/MORF complex together with KAT6B, which is responsible for acetylation of a substantial portion of H3 (39). Expression of both members of the MOZ/MORF complex (KAT6A and KAT6B) was increased in arousing hamsters (Chapter 2), which corresponds to the increased acetylation of H3 in arousal (Chapter 4). KAT13D is a core component of the circadian clock. It was recently described that liver does not retain circadian rhythm throughout hibernation, in contrast to the brain (40). Thus, the downregulation of KAT13D may reflect a halt in circadian rhythm in the liver of the torpid hamster.

Taken together, we found that overall RNA transcription is reduced during torpor and restored during arousals. Regulation of transcription occurs on the level of H3 acetylation through upregulated HDAC activity. Which HDAC and H3 amino acid are responsible for the upregulation of transcription in arousal, has yet to be studied.

MAPK PATHWAY IS INVOLVED IN CELL CYCLE REGULATION DURING HAMSTER HIBERNATION

In concert with the metabolic suppression, reversible cell cycle arrest has been reported in torpid ground squirrels (41,42). Cell cycle arrest may allow cells to withstand stress conditions and expand their life-span.

The MAPK pathway is involved in both immune system and cell cycle regulation through use of phosphatases, including the ERK1/2 cascade and the PP1 pathway (43). A suppression of MAPK corresponds to a suppression in transcription of e.g. Cyclin D which consequently leads to cell cycle arrest. In a study in ground squirrels a reduction of Cyclin D and Cyclin E protein in liver was found during torpor which was suggested to lead to a halt in G1/S phase of the cell cycle (42). During hibernation, MAPK inhibitors such as DUSPs and SPRYs are upregulated (Chapter 1), resulting in a downregulation of the MAP kinases, thus resulting in cell cycle arrest. Furthermore, we found that expression of TFs for cell cycle regulation was downregulated in torpor, such as JUN and FOS and
resume transcription in arousal (Chapter 1). Both JUN and FOS activate MAPK activity and therefore cell cycle progression. Furthermore, analysis of Transcription Factor Binding Site (TFBS) showed upregulated expression of three transcription factors in promoters of differentially expressed genes in arousal: EGR1, MNT and MYC. Interestingly, all three are regulated by members of the MAPK pathway and involved in the regulation of cell cycle, particularly through overexpression of the MYC gene which induces tumorigenesis. In-activation of Myc depletes histone marks in the genome that are associated with gene transcription (H3K9 acetylation and H3K4 methylation) (44). The reduction of H3K9 acetylation as found in Chapter 4 could be explained by a potential reduction of MYC in torpor and a delayed response of MYC activation in arousal. Moreover, MYC also recruits HATs to target chromatin and locally promote hyper-acetylation of multiple lysines on histones H3 and H4, which is in line with our increasing H3ac levels in arousing hamsters (Chapter 4) (45). Altogether, our studies suggest a role for MAPK pathway in the regulation of the cell cycle. Upregulation of DUSPs and SPRYs in torpor inhibit MAPK, resulting in cell cycle arrest, whereas TFs including Myc are activated in arousal, resulting in cell cycle progression. The regulation of cell cycle is possibly able to protect (hepatic) cells against stress throughout hibernation.

THE MAPK PATHWAY AND NFκB SIGNALING REGULATE THE IMMUNE RESPONSE DURING HIBERNATION

Next to changes in metabolism, a reduction in immune response is seen in a number of hibernating species (46–48). In skeletal muscle of the hibernating (including torpor and arousal) squirrel, reduction of TNFα and NFκB has been described (49). A balanced immune response during hibernation warrants tight control of the intensity and duration of the response. Besides regulating the cell cycle, the MAPK pathway plays a critical role in the immune response by mediating cellular responses to various extracellular signals, including cytokines, growth factors, and stress signals. This pathway is activated through a series of protein kinases that ultimately lead to the phosphorylation and activation of downstream transcription factors, including NFκB (Nuclear Factor kappa B), which regulate the expression of genes involved in inflammation, immunity, and cell survival. The MAPK pathway is involved in the activation of immune cells, such as macrophages, dendritic cells, and T cells, and is essential for the proper functioning of the immune system in response to infection and tissue damage. The NFκB pathway and the MAPK pathway have several interactions, e.g. DUSP3 is known to inactivate NFκB signaling and IL-10 expression enhances DUSP1 expression (50). Accumulated evidence suggests that some of the DUSP proteins mitigate the intensity
and duration of T-cell activation signal elicited by T-cell receptor engagement, which may be of importance in T-cell activation during torpor and arousal (51). On the other hand, sustained T-cell activation induces overexpression of DUSP proteins in T-cells, especially those conveying deactivation of ERK, which then contributes to premature T-cell aging. As aforementioned, the upregulation of DUSPs in torpor (Chapter 2) results in a reduction of MAPK enzyme activity. Consequently, their negative feedback regulation of MAPK may, contribute to the suppression of immune response in torpor. We found that the downregulation of the immune system in the liver of the hamster is reflected in increased expression of TNFAIP3 in arousal, which is a negative regulator of the NFκB signaling pathway and could be a protective factor in the ischemia/reperfusion injury during torpor (Chapter 1). In Chapter 5, we identified a reduction of the expression of cytokines TNFα and IL-6 in winter conditions and arousal early respectively, which could be the result of decreased NFκB signaling. Further investigation is required to verify the involvement of MAPK enzymes in the modulation of the immune response.

**FUTURE PERSPECTIVES**

The molecular insights gained in Chapters 2-4 of this thesis represent the fundamentals of Syrian hamster hibernation. Many of the results derived from cross-omics opens new questions into the biology of hibernation and will aid in the understanding of safe metabolic suppression. Analysis of the transcriptome and proteome of hibernating hamster as described in Chapter 2 and 3 shows changes in gene and protein expression in the liver. However, these changes were only determined on a whole-genome and total protein level, i.e. all differentially expressed RNAs or proteins were considered equally important in regulation of hibernation physiology. Additional factors influencing gene and protein expression such as alternative splicing, alternative TSS and miRNAs were not investigated. Alternative splicing is a cellular process in which specific exons from the same gene are joined in different combinations, leading to an increased diversity of proteins. mRNA transcripts originating from the same gene could encode for proteins with profound differences in function. Emerging evidence shows that DNA methylation also regulates alternative splicing. Exons, especially splice sites, have higher levels of DNA methylation than flanking introns. It would therefore be of interest to investigate differentially methylated target genes found in the torpor vs arousing hamsters and determine whether these methylation sites are involved in alternative splicing. Interestingly, alternative splicing has been found to occur in conjunction to changes in circadian rhythm (52,53), affecting glucose metabolism through modulating insulin secretion (52). The role of alternative splicing in hibernation has only been investigated in the heart of the hamster (54). This
study revealed that alternative splicing underlies the differences in abundance of the functional Cold-inducible binding protein in torpid hamsters. Further research addressing alternative splicing effects on hibernation may expand our knowledge of the phenotypic responses involved in hibernation.

The differentially expressed genes identified in Chapter 2 could be validated through q-RT-PCR and immunoblotting. Alternatively, the rapid developments using CRISPR technologies also allow to modulate gene expression at will without introducing mutations in the underlying DNA sequence which could be used to validate the clinical relevance of target genes identified in Chapter 2 in the physiology of hibernation (55,56).

The interaction of the MAPK members and the transcription factors (including MYC and CREB1) could be investigated using DNA footprinting and electrophoretic mobility shift assays (EMSAs). Further characterization of phosphorylation cascades and their downstream factors, notably MAPK, in other hibernating species and organs is warranted to investigate the generalizability of our hamster liver results.

The histone modifications as seen in Chapter 3 were detected in liver of hamsters. It would be of interest to measure these modifications in plasma and investigate their role in the regulation of IL-6 expression. Previously, chromatin immunoprecipitation experiments showed that pan-HDAC inhibitor increased histone activation marks H3K4me3 and H3K9ac at IL-6 promoter regions (57). These experiments could be repeated in different phases of hibernation to further elucidate the role of HDACs in regulation of the immune system in hibernating animals, which could aid in the development of new drugs or drug targets in e.g. organ transplantation, where regulation of the immune system plays an important role in the health and longevity of the transplanted organ.

In Chapter 4 we found differential expression of several HATs and HDACs, and stable HAT activity. In torpid hamster, we found that HDAC activity was increased in parallel to reduced H3 acetylation, suggesting a regulatory activating mechanism. To avoid energy expenditure by transcription of genes, histone deacetylases (HDACs) silence genes through chromatin remodeling. Previously, it was found that protein levels of HDAC1 and HDAC4 were significantly upregulated in 13-lined squirrels during torpor (4). Despite the suppression of a large amount of pathways in hibernating hamsters (13,58), our data and that of previous studies suggest the use of increased HDAC protein levels and activity in order to silence unnecessary genes in torpor.

Through inhibition experiments, we further elucidated that HDAC6 activity contributed the most prominently to differential HDAC activity. HDAC6 is a known deacetylase targeting lysine residues of both histones and (cytoplasmic) tubulin. Yet, the observed increase in alpha-tubulin levels in torpor are in stark contrast to the increased activity of HDAC6, suggesting that HDAC6 translocates from the cytosol to the nucleus in torpor. It would be of interest to measure HDAC6 activity in nuclear and cytosolic fractions or visualize HDAC6 translocation by immunostainings to further elucidate the role of
HDAC6 in hibernation of the hamster. Translocated HDAC6 plays a role in transcriptional regulation, which can be a useful mechanism to reduce metabolism of transplanted organs and protect the organ against ischemia-reperfusion injury.

Furthermore, we described the dynamic changes in the histone acetylome of the hibernating hamster (Chapter 4). Here only the histone acetylation of H3 and -4 were assessed. As described, histone modifications can present in many different forms, including methylation and phosphorylation of the histone tails. To broadly characterize post-translational histone modifications, LC-MS/MS or proteomics may be used to analyze other modifications than acetylation of the histone tails, which could reveal different mechanisms to regulate the metabolic suppression in hibernation. A study in ground squirrel showed that methyltransferase activity in liver was decreased at H3K4 and H3K9 residues during arousal, further supporting potential changes in lysine methylation (33). The differential expression of specified H3 lysines in Chapter 4 is suggested to change the chromatin condensation, which could be further investigated using techniques such as ATAC sequencing. Of particular interest would be assessing the activity of the MOZ/MORF complex, a histone acetyltransferase complex with a high affinity to the H3 lysines. The interaction of this complex with the histone complex could possibly be underlying the halt in cell cycle as described in a review by Dias et al. (41).

In Chapter 5 we showed that plasma of summer euthermic animals rescues the functionality of neutrophils in arousal early, however, further research is needed to identify the factors that underly this rescue mechanism. Through heat-inactivation and protease treatment of the plasma, we found that plasma from arousal early hamsters likely contains a humoral, heat-stable and protease resistant factor. The delayed expression of secondary response genes, including the increase in expression of IL-6 in arousal early hamsters, are likely governed by epigenetic regulation, but this assumption is yet to be confirmed.

CONCLUSION

Altogether, we identified changes in multiple processes during hibernation of the Syrian hamster. Firstly, we confirmed a change in metabolism from glucose metabolism to fatty acid oxidation. Secondly, components of the pentose phosphate are upregulated in torpid hamsters, which could aid in replenishing nucleotides for transcription and fuel for the TCA in arousal. The replenishing of the nucleotides in arousal is in line with the reactivation of transcription during arousal. We here identified increased activity of HDAC6 in torpor, which might play a role in the suppression of transcription during hibernation, in line with other epigenetic factors such as reduced H3 acetylation in torpor. Lastly, the MAPK pathway plays a significant role in hibernation of the Syrian hamster, regulating the turnover of the cell cycle and aids in suppression and reactivation of
the innate immune system. Collectively, hibernation is highly dynamic, which warrants closer examination of transition states at higher spatial and temporal resolution. Further identification of the transition states and protection against oxidative damage will aid to identify targets for protection of ischemia-reperfusion injury in several clinical settings, including, but not limited to cardiac arrest and organ transplantation. This thesis suggests a role for epigenetic regulation in metabolic and immune suppression. Using techniques such as Epigenetic editing, we will be able to mimic hypometabolism and/or protective mechanisms of the hibernator in a clinical setting, thus protecting organs from ischemia-reperfusion injury.
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General Discussion


