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# GENE EXPRESSION DURING SLEEP, WAKEFULNESS AND SLEEP DEPRIVATION

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## 1. ABSTRACT

Studies conducted on genes expression through the sleep-wake cycle and after sleep deprivation have revealed a number of genes that are more active during wakefulness/prolonged wakefulness than sleep, but also a few genes that are more active during sleep than wakefulness. Gene expression profiles during a specific vigilance state can be effectively monitored using microarrays, and when comparing profiles under different vigilance states, state-specific gene expression patterns can be precipitated. As such approach is random, and does not include any assumptions as to which genes would be expressed, it offers a non-biassed view on the participation of different factors in the regulation of vigilance states. Combining results from different species offers a powerful tool for the analysis of genes that are involved in sleep-wake cycle regulation. The results of such assays are dependent on what area the tissue for the analysis is collected and the time point of the collection in respect to the duration of the specific vigilance state.

## 2. INTRODUCTION

### 2.1. What is gene expression

Gene expression describes the transcriptional activity of the cell, and is measured by quantifying the amount of messenger RNA. As messenger RNA is synthetised in preparation to new protein synthesis, the amount of mRNA can be regarded as a rough measure of synthetic activity of the cell. Depending on the chosen method, the expression of one (*in situ* hybridisation) or several (RT-PCR, gene arrays) genes can be measured simultaneously.

The amount of mRNA is regulated by the rates of synthesis and degradation, and thus not only an increase in synthesis rate will result in higher mRNA levels, but also decrease in degradation rate (stabilization of the mRNAs). It can be further noted that transcription will not always lead to translation (synthesis of the coded protein).

An alternative for gene expression measurement is to measure the concentration of the product - e.g. enzyme, transporter, neurotransmitter. The advantage of the mRNA measurement is that either no previous information concerning the molecules that are affected (random search) or only knowledge of the gene sequence (*in situ* hybridization, RT-PCR) are required.

### 2.2. What can gene expression tell us about sleep

The main reason to measure gene expression through sleep-wake cycle and/or during sleep deprivation is to find out which genes are activated/deactivated during a specific vigilance state, and thus may be involved in the regulation of sleep. One of the aims is to find “sleep genes” - genes, whose activation promote a specific sleep state. This knowledge would be helpful in trying to understand the purpose of sleep. It should be noted, that “sleep genes” can, and have been detected by other genetic approaches, described in more detail elsewhere in this book (see Tafti & Franken).

The several hypotheses concerning the meaning of sleep may get clarification about knowledge of the genes that are activated/deactivated through sleep. The association of genes that regulate energy metabolism strengthen hypotheses about sleep being associated with energy need, while genes associated with plasticity give support to the hypothesis that sleep is needed for learning and memory. Most of the genes that so far have been found to change expression according to vigilance state have not given any direct answer to the question concerning the purpose of sleep. Immediate early genes, as well as transcription factors are involved in myriads of intracellular signalling pathways. However, in the long run the accumulation of knowledge will help to elucidate the exact role of each of these factors.

The executive mechanisms of sleep are of equal interest as the purpose of sleep. The already existing knowledge of these mechanisms promotes potential sleep genes for evaluation. The secretion patterns of several neurotransmitters in the course of vigilance state changes are well characterized. Several other molecules, including growth hormone axis hormones and cytokines, are known to undergo changes in the course of sleep-wake cycle. The expression of the genes that regulate the synthesis of these molecules have been recently characterized.

The immediate early gene c-fos has also been used as a marker of neuronal activity: cells that are active during waking, express more c-fos during waking, while sleep-active cells express c-fos during sleep (1). The site of activation may reveal sites that previously have not been known to regulate vigilance states. The best known example of this is the discovery of the sleep active cells in the ventrolateral preoptic area of the hypothalamus (VLPOA) (1).

### 2.3. What gene expression cannot tell us about sleep

Increased gene expression can be regarded to reflect increased need to synthesise the target protein. Synthesis of proteins as response to external or internal signals is a complicated process involving easily hundreds of molecules. The intracellular signalling chains as well as the protein synthesis machinery itself use the same molecules for synthesis of different molecules. Thus the increase in expression of these molecules is a relatively nonspecific signal. However, if an expression profile, using e.g. microarrays, can be established, it is at least theoretically possible to reconstruct the signalling and synthesis pattern of an individual molecule.

The relationship between gene expression and increase in the actual protein amount may be complicated, and time delayed, and some times increase in mRNA does not result in synthesis of the corresponding protein. It will be important to measure both gene expression and the concentrations of the end product to get a more detailed view of the behaviour of the molecule of interest in the course of sleep-wake cycle and sleep deprivation.

It should be noted that an activation of any system can take place without subsequent increase in gene expression based e.g. on phosphorylation and dephosphorylation of molecules. Thus negative results in gene arrays are not conclusive. Moreover, if the gene codes for a transcription factor or immediate early gene, the final target gene and its protein are not known. The immediate early gene c-fos was among the first detected genes whose expression was measured in connection with sleep-wake cycle. The expression can be used as a measure of activation of cells, but the target gene(s) that code for the transcription of biologically active protein(s) is not known.

It can also be noted that the first sleep related gene so far, orexin/hypocretin, is not activated during sleep deprivation, but does have diurnal variation in gene expression, indicating more relation to circadian system than to sleep inducing effects, or sleep regulation (2).

## 3. STRATEGIES TO STUDY GENE EXPRESSION

### 3.1. Random search

The basic idea of this type of analysis is to extract the total RNA of the brain area of interest from animals that have been handled differently (e.g. at different times of the sleep-wake cycle, or after sleep deprivation), and compare the gene expression profiles between the handlings. The mRNA:s that are common to both conditions are ignored, while the mRNA:s that are expressed differently under the different conditions are analysed further. As many mRNA:s (from tens to hundreds) are found, the analysis will be tedious and time consuming. Mostly the differences are not considerable, and the analysis must be repeated several times in order to eliminate random changes. The main methods used are subtraction library, which was the first method applied to sleep research (3) and differential display (4). Recently commercially available cDNA microarrays have made it possible to analyze simultaneously the expression of thousands of genes from one sample. The efficacy of this method allows repeated analyses to exclude random effects, and when similar analyses are performed across species, interesting results may be obtained. E.g. if the same genes are more active in sleep in fruit flies, hamsters and rats, we have every reason to hypothesize that these genes have a strong connection to sleep regulation.

If a random search in repeated runs shows increased/decreased mRNA amounts of a gene during e.g. sleep deprivation, the gene needs to be identified and possibly cloned, if it is not previously known. When the gene sequence is known, probes for *in situ* hybridization and RT-PCR can be prepared to confirm the changes in gene expression. The confirmation by independent methods further decreases the chance of a false positive finding. If the mRNA change can be found with independent methods, and also across several species, we can be rather confident that this specific gene is activated/deactivated as response to change in vigilance state.

### 3.2. Targeted search

In cases when it is already known, or there is a good reason to hypothesize that a substance is involved in regulation of sleep-wake cycle, or may be a homeostatic

regulator of sleep, gene expression of the molecules that are involved in its metabolism (e.g. rate limiting enzymes, receptors and transporters) can be measured. The methods of choice are Northern blott analysis, *in situ* hybridization or RT-PCR (reverse transcriptase polymerase chain reaction). The methods are based on targeting the gene of interest using specific probes, and amplifying the signal. The Northern blot analysis and RT-PCR use tissue extracts. In the RT-PCR reaction the mRNA is first transcribed to cDNA using reverse transcriptase, and the cDNA is amplified and detected on gel. The method is very sensitive, but quantitation is challenging. *In situ* hybridization is performed on tissue sections. A radioactivly labelled probe adheres to the target gene and can be visualized using photographic emulsion or film. The advantage of this method is, that the information of the anatomy of the gene expression is preserved. *In situ* hybridization signal can also be quantified, but only under carefully controlled conditions. For more detailed description of the methods, see reference (5).

### 3.3. Map cell activity

The expression of the immediate early gene c-fos and/or its product, c-Fos protein, has been successfully used to identify cell activity during different vigilance states. Below are provided some examples of the different strategies that have applied the c-fos/c-Fos expression.

The finding of the sleep active cells of the ventrolateral nucleus (VLPO) was based on the expression profile of fos: C-Fos expression was measured in different brain areas during wakefulness and sleep as well as sleep deprivation and recovery sleep. It was found that a small number of cells in the ventrolateral hypothalamus showed more c-Fos expression during sleep than wakefulness (1).

C-fos expression in the cholinergic cells of the vertical and horizontal limbs of Broca was higher during wakefulness than sleep, while the cholinergic cells of the medial septal nucleus did not change their c-fos expression according to vigilance state (6). In double labelling studies with c-Fos and different neurotransmitters it has been found that during recovery from REM sleep deprivation the number of c-Fos/ChAT and c-Fos/GAD positive neurons increased, while that of the c-Fos/Ser and c-Fos/TH decreased (7).

The number of orexin cells expressing c-Fos was higher in rats during the dark period than during the light period. Sleep deprivation also increased Fos expression in orexin neurons (8).

Changes in c-Fos expression as response to prolonged wakefulness were compared in young and old rats. The increase in c-fos expression was significantly lower in the old than young animals (9).

## 4. WHERE TO SEARCH

One factor to complicate the gene expression analysis is the choice of the brain site from where the tissue is collected. There are several specific nuclei in the brain that are known to regulate sleep, e.g. the monoaminergic cell groups, the cholinergic cell groups in the basal forebrain and pons as well as the GABAergic neurons in the hypothalamus, or the orexin cells in the lateral hypothalamus, not to forget the reticular nucleus of the thalamus (for review see (10)). However, the phenomenon of sleep is measured as electrical changes in cortical activity, and the cortex can be described as target site of the sleep/waking active neuron groups.

Most of the existing data concerning gene expression changes in the course of sleep/wake cycle and sleep deprivation has been collected from cortex. It is probable that the results would be different if tissue collection would have taken place from other brain areas.

This is evidenced already in the first subtraction library work (11), where after a 24 h sleep deprivation 2 mRNA transcripts were found that were affected by sleep deprivation. These transcripts coded for proteins neurogranin and dendrin. The mRNAs for neurogranin and dendrin were decreased in subcortical forebrain and midbrain areas, while there was a tendency to increases in the hippocampus (3,11). In another study, sleep deprivation for 3 and 6 hours increased A1 receptor mRNA in the basal forebrain while it had no effect in the cortex (12).

## 5. WHEN TO COLLECT THE TISSUE

Presently the sample for gene expression studies can be collected only at one time point from one animal. Transition from one vigilance state to another is a dynamic process making it difficult to evaluate at which time point into the state the sample should be collected. The expression profile of genes changes rapidly also in the course of sleep deprivation, as the signalling pathway cascades proceed. E.g. immediate early gene expression will be eventually followed by the expression of the target gene. As several cascades are simultaneously activated, some of them using the same intracellular messengers, it is not easy to figure out the sequence of events, or the specific pathways that are involved.

The effect of time is clearly evident in a study which showed highest number of c-Fos immunostained cells in several brain areas after 3 h of sleep deprivation, but the number had started to decline at 6h. In two brain areas the expression was higher at 6h after the deprivation than at 3h (13). This study demonstrates clearly not only the importance of the time factor but also the regional differences in expression profiles.

Unfortunately, the conclusion is that to obtain even an approximate idea of gene expression during vigilance states and sleep deprivation, tissue has to be collected at several time points. Analysis of these data using even the rapid methods of cDNA arrays is a major task.

**Table 1.** Table of genes that change their expression during wakefulness/prolonged wakefulness

| | Brain area | Method | SD effect | Reference |
|---|---|---|---|---|
| **Neurotransmitter systems** | | | | |
| Adenosine A1 receptor | BF | *in situ*, RT-PCR | 3 and 6h SD ? | (12) |
| Adenosine A2a receptor | olf. tub. | *in situ* | 3 and 6h SD ? | (12) |
| Adrenergic α1A receptor | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Adrenergic β2 receptor | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| AMPA glur2 receptor | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| AMPA glur3 receptor | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Aryl sulfotransferase | cortex | DD, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Chromogranin C | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| GABA-A β3 receptor | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| GLAST 1 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Muscarinic m1 receptor | brain | *in situ* | REMSD- | (28) |
| Muscarinic m2 receptor | pons | *in situ* | REMSD ? | (28) |
| Muscarinic m3 receptor | pons, BF | *in situ* | REMSD ? | (28) |
| NET | LC | RT-PCR | REMSD ? | (90) |
| Nicotinic β2 receptor | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| NMDA 2A receptor | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| NTT4/Rxt1 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Tyrosine hydroxylase | LC | *in situ* RT-PCR | TSD, REMSD ? | (89,112,124) |
| **Energy metabolism** | | | | |
| COX subunit I | cortex | DD, RPA, RT-PCR | 3h wake, SD? | (4) |
| Glut 1 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Glycogen synthase | cortex | Northern blot | TSD 6h? | (45) |
| Glycogen phosphorylase | cortex | Northern blot | TSD 6h? | (45) |
| NADH dehydrog. subu.2 | cortex | DD, RPA, RT-PCR | 3h wake, SD? | (4) |
| PTG | cortex | Northern blot | TSD 6h? | (45) |
| 12S rRNA | cortex | DD, RPA, RT-PCR | 3h wake, SD? | (4) |
| Vgf | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| **Immediate early genes and transcription factors** | | | | |
| c-fos | brain | *in situ* RT-PCR, Northern blot | 3 and 8h wake, TSD? | (37,69,71) |
| CHOP | cortex | DD, cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| IER5 | cortex | DD, RPA, RT-PCR | 8h wake, TSD ? | (4) |
| JunB | brain | Northern blot | TSD? | (37) |
| NGFI-A | brain, cortex | DD, cDNAarray, RPA, RT-PCR, *in situ* | 3 and 8 h wake, TSD ? | (18,37,85) |
| NGFI-B | cortex, brain | cDNAarray, RPA, RT-PCR Northern blot | 8 h wake, TSD ? | (4,37) |
| **Neuropeptides** | | | | |
| Galanin | hypothalamus | *in situ* | TSD-, REMSD? | (52) |
| GHRH | hypothalamus | *in situ* | TSD?, REMSD? | (57,58,130) |
| Somatostatin | hypothalamus | *in situ* | TSD?, REMSD? | (57,130) |
| **Heat shock proteins, chaperones** | | | | |
| BiP | cortex | DD, cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| ERP72 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| GRP75 | cortex | DD, cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| HSP60 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| HSP70 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| **Neural plasticity related** | | | | |
| Arc | cortex | cDNAarray, RPA, RT-PCR | 3 and 8h wake, TSD? | (4,18) |
| BDNF | cortex hippocampus | DD, RPA, RT-PCR, RPA | 8h wake, TSD? 8h TSD - | (4,18) |

| | | | | |
|---|---|---|---|---|
| Dendrin | forebrain | subtr, lib., *in situ* | 24 h TSD? | (3) |
| F3 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| MMP-9 | cortex | RT-PCR | 8h TSD ? | (18) |
| | hippocampus | | 8h TSD? | |
| Neurogranin | subcortex,cortex | subtr, lib., *in situ* | 24 h TSD? | (11) |
| Synaptogamin IV | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| tPA | Cortex | RT-PCR | 8h TSD ? | (18) |
| | hippocampus | | 8h TSD - | |
| TrkB receptor | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| **Others** | | | | |
| Calmodulin | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Cyclin D2 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Interleukin-1? | hypothalamus, BS | RT-PCR | TSD? | (67,68) |
| JNK1 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| LMO-4 | cortex | DD, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Metallothionein 3 | cortex | DD, RPA, RT-PCR | 8h wake, TSD? | (4) |
| N-ras | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| PTP1B | | | 8h wake, TSD? | (21) |
| Rlf | cortex | DD, RPA, RT-PCR | 3h wake, SD? | (4) |
| SGK1 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| Stat3 | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |
| TIMP-1 | | | 8h wake, TSD? | (21) |
| Tr? | cortex | cDNAarray, RPA, RT-PCR | 8h wake, TSD? | (4) |

It should be noted that the functional groups are to some degree speculative since the function of several genes is presently incompletely understood. DD= differential display, *in situ= in situ* hybridization, REMSD=REM sleep deprivation, RT-PCR=reverse transcriptase polymerase chain reaction, RPA=ribonuclease protection assay, subr.lib.=subtraction library, TSD=total sleep deprivation.

## 6. WHAT HAS BEEN FOUND

### 6.1. Waking genes

Most of the genes that have been found to be related to sleep/wake cycle are more actively expressed during wakefulness than during sleep. After 24h sleep deprivation 10 transcripts of the screened 4000 clones were affected by SD, while in another study 44 of about 10 000 were elevated after waking/sleep deprivation (4). In the latter study, only 10 genes increased their expression during sleep.

Below genes that have been found to be effected by wakefulness and/or prolonged wakefulness are presented in alphabetic order. See also Table 1.

**Adenosine receptor $A_1$** was upregulated by 3h sleep deprivation in the basal forebrain, while the **$A_{2A}$ receptor** gene expression was induced in the olfactory tubercle (12).

Adenosine $A_1$ receptor mediates the inhibitory effects of adenosine, while $A_{2A}$ receptor is excitatory (14). $A_1$ receptor is widely expressed in the CNS, and the effects of adenosine on vigilance state regulation appear to be mediated by that receptor subtype (12,15,16). Extracellular adenosine levels increase locally in the brain in the course of prolonged wakefulness (17). It may be speculated that the potential increase in A1 receptors would further strengthen the effects of adenosine during sleep deprivation.

**Adrenergic receptors $alpha_{1A}$ and $beta_2$** were upregulated after 8h waking and sleep deprivation (4).

The receptor $alpha_{1A}$ is an inhibitor autoreceptor, which decreases the release of noradrenaline to the synaptic cleft as response to stimulation while $beta_2$ is a stimulatory receptor. Both receptors are widely expressed in the body, including CNS.

**Activity-regulated cytosceleton-associated protein (Arc)** (= Arg 3.1) was increased after 3 and 8 h of waking and sleep deprivation (4,18).

Arc, an effector early gene, is strongly induced by patterns of synaptic activity that also induce long-term potentiation. Interestingly, the induced arc mRNA is delivered to dendrites that experienced neuronal activation. This targeting requires NMDA receptor activation (19). Thus arc can be grouped also to the genes that are associated with neural activation and neural plasticity. The expression of Arc has been found to be high also during spontaneous waking (20).

Arylsulfotransferase (AST), an enzyme that catabolizes catecholamines, was upregulated during prolonged wakefulness in several species (4). The degree of expression appears to be directly proportional to the duration of the previous wakefulness (21), unlike in most of the waking related genes. It may be speculated that the increase in gene expression is associated with the increased activity of the noradrenergic system during prolonged wakefulness.

Brain derived neurotrofic factor (BDNF) gene expression increased after 8h waking and sleep deprivation (4,18).

Neurotrofins regulate cell differentiation, survival and neural plasticity (22). BDNF binds to and activates the

TrkB tyrosine kinase receptor (mRNA also increased after 8h of sleep deprivation) (23). The expression of BDNF has been measured to be high during waking (20)and sleep deprivation (4). However, after REM sleep deprivation for 6h the immunoreactivity for BDNF was found to be lower in the brain stem and cerebellum while there was no change in the hippocampus as compared to controls (24). A systematic study of both gene expression and immunoreactivity at several time points during spontaneous wakefulness and sleep as well as in the course of sleep deprivation/specific REM sleep deprivation will be required to clarify the role of BDBF:s in sleep regulation.

**BiP** expression was increased after 8h waking and sleep deprivation (4).

BiP is a member of the heat shock protein family (see heat shock proteins). It is an eucaryotic Hsp70 homologue, which interacts with the substrate molecules in an ATP-dependent manner, as the Hsp70 family (25). BiP is the major chaperone of the endoplastic reticulum, and it is $Ca^{2+}$ -regulated.

**Calmodulin** gene expression increased after 8h waking and sleep deprivation (4).

Calmodulin is the main intracellular calcium concentration sensor and regulator. It is activated upon intracellular $Ca^{2+}$-release, which can be induced through several intracellular signalling cascades preceded by depolarization or receptor binding, and interacts with its target molecules. As target molecules calmodulin has protein kinases and phosphatases, adenylyl cyclases, ion channels, receptor proteins and NO synthases (26). Thus neural activation is frequently associated with calmodulin binding, and it can be speculated that increased neuronal activity that is associated with prolonged wakefulness would induce increased expression of the calmodulin gene.

**Chaperons**

Chaperons are molecules that are related to the folding of newly synthetised proteins in the cell. Protein folding inside cells is not a spontaneous process, but is an energy consuming process that involves proteins that actively aid the folding procedure, at least partly by preventing the aggregation of the newly synthetised protein chain (27). Many chaperons are synthesized as response to stress.

**Cholinergic receptors**

Cholinergic receptors are widely distributed in the body, including CNS. Both muscarinic (M1-5) and nicotininc (alpha$_{1-8}$ and beta$_{1-5}$) receptors are found in the CNS. a) The expression of muscarinic receptors M3 was found to be increased after 72h REM sleep deprivation in the pontine nuclei and nucleus accumbens and bed nucleus of stria terminalis, while there was no change in m1 receptor expression. M2 receptor expression was decreased in the pontine nuclei (28). b) Nicotinic receptor subunit beta**2** was upregulated after 8h waking and sleep deprivation (4).

**CHOP =c/EBP** homology protein gene expression was increased after 8h waking and sleep deprivation (4). CHOP is a non-ER (ER= endoplasmic reticulum) bound transcription factor that is induced by a variety of adverse physiological conditions, including ER stress. Accumulation of unfolded proteins in the ER activates a response that targets both ER bound chaperons (e.g. BiP) and CHOP (29). CHOP expression is associated with cell stress and apoptosis (30).

**Chromogranin C** was induced by 8 h of waking and sleep deprivation (4).

Chromogranin/secretogranins are a family of proteins with a widespread distribution in secretory vesicles(large dense core vesicles) of endocrine and nervous tissues. Secretogranin II (chromogranin C) responds to both short (stimulation induced seizure) and long term stimulation (e.g. water or food deprivation) by increasing gene expression (31).

**C-fos** expression was found to be increased after 24 h sleep deprivation in several brain areas of rats (32)and in the preoptic area of cats (33), and in the brain stem after prolonged periods of REM sleep (34).

C-fos is the classical immediate early gene, induced by a variety of stimuli. The immediate early gene response is rapid (gene expression is increased within 20 min upon stimulation) and transient - even the protein disappears within a couple of hours after the stimulation.

C-fos expression was shown to have a circadian pattern (35)and to be lower during spontaneous sleep than waking (35,36).

**C-jun** mRNA was not increased by sleep deprivation (37), while the **c-Jun NH2-terminal kinase (JNK1)** gene expression was increased after 8 h waking and SD (4). The JNK/c-jun signalling pathway is activated by cytokines and stress stimuli (38). Activated jun can lead to either apoptosis, as evidenced in several neuronal cell lines (39), or it may promote cell survival and development in other cells (40). Thus the response to JNK/c-jun activation is dependent on the cell type, and may be either harmful or useful.

**Cytochrome C subunit 1 (COX1)** gene expression was upregulated after 3h waking and sleep deprivation (4).

Cytochrome C oxidase is the inner mitochondrial membrane enzyme that catalyses the last step in the electron-transfer chain. This reaction chain is essential for energy (ATP) production in all cells. Interestingly the expression of this gene is upregulated only after 3h of wakefulness - after 6 hours he expression is no more elevated suggesting that other mechanisms to compensate for increased energy need have taken over.

**Cyclin 15 D2 (vin-1)** gene expression was increased after 8h waking and sleep deprivation (4).

Cyclins, activated by mitogenic signals, are positive regulators of the cell cycle, and their activation precedes cell proliferation (41). Several cancer types involve changes in cyclin 2 expression. It can be noted that c-jun NH2-terminal kinase 1 appears to participate in the signalling pathway that controls the expression of cyclin D2 (42).

**Dendrin** expression was decreased after 24 h sleep deprivation in the subcortical areas and midbrain (3).

Dendrin is expressed exclusively in forebrain structures, where the mRNA is present in neuronal cell bodies and dendrites, while the polypeptide is localized almost exclusively in the dendrites. It has been suggested that dendrin has a role in synaptic plasticity of neocortical forebrain neurons (43).

**6.2. Energy metabolism**

In accordance with the view that sleep propensity is regulated by energy metabolism during (prolonged) wakefulness, several genes that regulate molecules of energy metabolism have been found to be upregulated during (prolonged) wakefulness, including cytochrome C subunit 1 (COX1), NADH dehydrogenase subunit 2, glucose transporter type 1 (GLUT1) and Vgf (21). Several interesting notions can be made concerning these genes. The mitochondrial proteins COX and NADH dehydrogenase are part of the electron transfer chain located in the inner mitochondrial membrane. Their synthesis is coded by the mitochondrial, not by the nuclear genome. The mitochondrially coded subunits respond more readily to the increased energy need by increasing transcription (44). It is also very interesting to note, that the upregulation of these genes took place after only 3h of sleep deprivation, and was no longer present after 8 h of sleep deprivation, indicating a rapid response to increased energy need. However, a more long lasting increase in energy need must be regulated by methods that do not involve increased gene expression of these two mitochondrial genes.

The genes of glycogen metabolism were also affected by prolonged wakefulness. It has recently been shown that a 6h sleep deprivation in mice increases the expression of protein targeting to glycogen (PTG) and decreases the expression of mRNAs encoding glycogen synthase and glycogen phosphorylase (45). These results give support to the hypothesis that glucose metabolism would be associated with the regulation of sleep propensity (46).

In addition to the role of glucose transport through the blood brain barrier by glut 1 transporter, the increased expression of this transporter may emphasize the role of astrocytes in the regulation of energy balance (47).

**ERP72** gene expression was increased after 8 h waking and sleep deprivation (4).

ERP72 is belongs to ER luminal proteis, and acts as a chaperon protein. Its expression in the brain is induced e.g. by ischaemia (48).

**F3** gene expression was increased after 8h waking and sleep deprivation (4). Neuronal (axonal) adhesion glycoprotein F3 is a member of the immunoglobulin superfamily (49). It influences axonal growth and fasciculation. High levels of F3 expression were found in the hypothalamo-neurohypophysial system, and it has been suggested that F3 promotes remodelling of neurosecretory terminals (50). Another suggested role for F3 is functioning as signalling molecule in communication between neurons and glia during neuronal growth (51).

**GABA receptor GABA-A$_3$** gene expression was upregulated after 8h waking and sleep deprivation (4).

GABA is the main inhibitory neurotransmitter in the CNS. GABA-A receptor is a $Cl^-$ channel, and its opening decreases depolarization. The GABA-A receptor family consists of 21 cloned subunits genes (?alpha$_{1-6}$, ?beta$_{1-4}$, gamma?$_{1-4}$, sigma$_{1-3}$, delta?, pii, theta and epsilon?), of which one (3) was found to be upregulated during sleep deprivation.

**Galanin** A 24 h REM sleep deprivation increased galanin mRNA in the periventricular nucleus and the medial preoptic area of the hypothalamus (52).

Galanin is an inhibitory neuropeptide (53). It is widely expressed in the CNS, and has been found to participate in many brain functions, such as feeding, memory, nociception and endocrine activity (53). Galanin is located in the brain areas that are known to regulate sleep: preoptic area, hypothalamus and brain stem nuclei (54,55). It is of interest to note that in the rat 90% of the GABAergic sleep-active cells in the ventrolateral preoptic nucleus (VLPOA) are also galaninergic (56).

The number of cells expressing **GHRH** in the paraventricular nucleus was lower after 24 and 72 h REM sleep deprivation, while the number of cells expressing SRIF (somatostatin) was increased in the arcuate and periventricular nuclei (57). The expression of GHRH (growth hormone releasing hormone) displays a diurnal rhythm measured in the cortex and hypothalamus of rats with highest mRNA levels around the light onset. The levels declined during the light period and stayed low in the dark (58). For more details discussion of GHRH, see Obál and Krueger.

**Glucose transporter Glut 1** was upregulated after 8h waking and sleep deprivation (4).

Glut 1 belongs to the family of glucose transporters Glut 1-5, 7 and 8 (59). Of these transporters Glut 1, 3 and 4 are found in abundance in the brain.

Glut 1 is the major glucose transporter of the blood brain barrier and is present in glia while glut 3 is found in neurons (60). Alterations in energy demand affect the expression of the glucose transporters (61).

**Glutamate/aspartate transporter (GLAST)** gene expression increased after 8h waking and sleep deprivation (4).

Glutamate/aspartate transporter,GLAST, is one of the five excitatory amino acid transporters (EAAT). Two of the five, GLAST (EAAT-1) and GLU-1 (EAAT-2) are predominantly expressed in astrocytes, and seem to be the main glutamate transporters in the brain (62). After neuronal firing the released glutamate is taken up by GLAST and GLU-1 to astrocytes to avoid the cytotoxic effects of high glutamate concentrations (63).

**Glutamate receptors** (ligand gated ion channels) NMDA 2A, AMPA Glur 2 and Glur3 are upregulated after 8h waking and sleep deprivation (4).

Of the cloned 16 ionotropic glutamate receptors three (NMDA 2A, AMPA Glur 2 and Glur3) were found to increase mRNAs during sleep deprivation in the brain.

**Glycogen metabolism** 6h sleep deprivation in mice decreased the mRNA of **glycogen phosphorylase** and **glycogen synthase**, the rate limiting enzymes of gylocogen metabolism (45), while the activity of the glycogen synthase was increased 2.5 fold. Glycogen phosphorylase catalyses phosphorylysis of glycogen, and is the first step in the reaction chain to produce glucose from glycogen. Glycogen synthase is the enzyme in the glycogen synthesis chain that adds one glucose molecule to the glycogen chain.

**Protein targeting to glycogen (PTG)** interacts with the above mentioned enzymes, and sets glycogen metabolism in a synthetic mode. The expression PTG increased two-fold after sleep deprivation (45).

**6.3. Heat shock proteins**

The gene expressions of HSP60, HSP70 and GRP75 were increased after 8h waking and sleep deprivation (4). Heat shock proteins are classified according to their molecular weight (HSP 32, HSP60, HSP70, HSP90). Hsp70 is the main inducible HSP family, and it consists of constitutively expressed members (Hsc70) and stress inducible forms (Hsp70). Glucose-regulated protein, GRP75, is a member of the Hsp 70 family, and it is localized in the mitochondria.

Heat shock response is one of the most primitive and highly conserved cellular defence systems known in biology. The response is triggered by several stressful stimuli, including high temperature, heavy metals, oxidants, endotoxins and ischaemia/reperfusion. The response protects against acute cell injury, e.g. apoptosis (64). Heat shock proteins are found in all cellular compartments, including cytosol, mitochondria and endoplastic reticulum.

**IER5** gene expression was induced after 8h waking and sleep deprivation (4).

IER5 is a member of slow-kinetics immediate early gene family, that can be induced by serum- and growth factors. Based on the structure of the gene it has been speculated that IER5 may play a role in mediating the cellular response to mitogenic signals (65).

**Interleukin-1** is a member of a large group of cytokines that regulate immune responses during infection and inflammation.

IL-1? affects the thermoregulatory area in the preoptic area of the anterior hypothalamus and mediates the fever response. Injection of IL-1? increase non-REM sleep, which may partly explain the increased sleepiness during fever (66). A 24-h total sleep deprivation increased the amount of IL-1?mRNA in the rat hypothalamus and brain stem (67). IL-1? has also a diurnal rhythm of gene expression with maximal expression in the early part of the light phase (68). For more detailed discussion of interleukines see Obal and Krueger.

**Immediate early genes.** The first group of genes described to be expressed according to vigilance state were the immediate early genes (32,35,37).

Immediate early genes are transcription factors, which can be divided to a) inducible transcription factors: c-Jun, JunB, c-Fos, Fra-1, Fra-2, Krox20 (Egr-2), Krox24 (NGFI-A, Egr-1, Zif268) and b) constitutive transcription factors: CREB, CREM, ATF-2 and SRF (28).

Immediate early genes encode regulatory proteins that control transcriptional response of cells to external stimuli. Typically, immediate early gene expression occurs rapidly and transiently after the stimulus. Both spontaneous wakefulness and a 3- 24 h sleep deprivation increase the amount of c-fos mRNA and FOS protein in several brain areas, especially in the cortex, hypothalamus, thalamus and some brain stem nuclei (69-71). During very long periods of wakefulness (>24 h) the expression of immediate early genes is no longer elevated.

Jun and Fos proteins are induced and activated following most physiological and pathophysiological stimuli in the brain. They bind as different composition heterodimers in the AP-1 binding site to activate target gene expression. The immediate early gene **junB** was found to be expressed in rats more during the dark period, while c-jun remained unchanged through the day and in the course of sleep deprivation (37,72).

**LMO4** gene expression was induced after 8h waking and sleep deprivation (4).

LMO4 belongs to the LIM-only (LMO) group of transcriptional factors, that act as adapters in protein interaction in transcription. Recently it has been found that LMO4 expression is increased in breast cancer (73).

**Metalloproteinase-9 (MMP-9)** gene expression was increased after 8 h sleep deprivation in the cortex but decreased in the hippocampus (18).

Metalloproteinases are zinc-dependent endopeptidases, which degrade components of the extracellular matrix, e.g. collagen and laminin. In the central nervous system MMP:s may contribute to neurite outgrowth but also to pathological events (74).

**Metallothionein 3** (=growth inhibitory factor GIF) gene expression was induced after 8 h sleep deprivation (4).

Metallothioneins (MT) are ubiquitous low molecular weight metal binding proteins that are involved

in regulation of small metal ion (especially zinc and copper) concentration in cells, and detoxification of heavy metal ions, specifically in liver. MT3 is specifically expressed in the cells of neural origin and is down regulated in Alzheimer's disease (75). It may act as antioxidant to protect against oxidative stress (76). Compared to other MT:s, MT3 has specific biding properties, that may be used for buffering zinc concentrations in zincergic neurons and for transfer of zinc to synaptic vesicles (75). MT3 inhibits neuronal cell growth (77). In the brain the zinc-containing neurons, that sequester zinc in their presynaptic vesicles and release it in a calcium- and impulse-dependent manner, are glutamatergic and can be found almost exclusively in the forebrain (78).

**NADH dehydrogenase subunit 2** expression was increased after 8h waking and sleep deprivation (4).

NADH dehydrogenase complex (Complex I) is the first step in the inner mitochondrial membrane electron transfer chain.

**Neurogranin/RC3** gene expression was decreased after 24h sleep deprivation in the subcortical forebrain and cortex (11). Neurogranin is a neuron specific protein that binds calmodulin and is activated by protein kinase C and $Ca^{2+}$. It is expressed richly in dendritic spines of hippocampal pyramidal cells (79). Neurogranin appears to have a function in LTP, and knock out mice lacking the neurogranin gene have deficits in hippocampal synaptic plasticity and spatial learning impairments (80).

**Nerve growth factor (NGF**). For this molecule antibodies have been available, and changes in NGF have been measured using them. Sleep deprivation for 6h increased the number of NGF immunoreactive neurons in the cortex (81), while a 6h specific REM sleep deprivation decreased NGF in the hippocampus but had no effect in the cerebellum and brain stem (24).

Nerve growth factor is the first neurotrophin that was invented. It has at least two important functions: to support mature neurons in a trophic manner, and target the innervation of sympathetic and sensory ganglia to target organs during development. NGF binds with high affinity to the TrkA receptor (see trk receptors), but can also bind to the low-affinity neurotrophin receptor, p75NTR (82). The cell survival signals of NGF are mediated through the trkA receptor, while binding to p75NTR receptor induces apoptotic cell death. In mature cells NGF signalling mediates neuroprotective and repair functions (83). It appears to have a role also in inflammatory and autoimmune states and nociseption (84).

**NGFI-A (=Egr-1, Krox-24 or Zif-268)** gene expression was increased already after 3 h waking, was maximally increased after 6h sleep deprivation, and had already decreased after 12 h dep (18,37,85).

NGFI-A is an inducible transcription factor (immediate early gene) that is activated with many stimuli, including mechanical injury (86). Interestingly, NGFI-A activation has been associated with the development of LTP and long term memory (87).

**NGFI-B (=TR3 or nur77)** gene expression increased after 8h waking and sleep deprivation (4,37).

NGFI-B belongs to the steroid/thyroid nuclear receptor superfamily, but its endogenous ligand is unknown - thus it is an orphan receptor. The receptor is upregulated by external stressors like seizures and ischaemia (88) as well as nerve growth factor *in vitro* (89). Unlike other transcription factors, NGFI-B can enter mitochondria, and there regulate the initiation of apoptosis (88).

Three to five days of REM sleep deprivation increased **noraderanaline transporter (NET)** mRNA in the locus coeruleus, which may indicate increased presynaptic efficacy in noradrenergic neurotransmission (90). NET is localized in the presynaptic terminals of noradrenergic cells, and accounts for the noradrenaline reuptake and maintenance of noradrenaline stores in the synaptic terminals.

**N-ras** gene was upregulated after 8h waking and sleep deprivation (4).

N-ras belongs to a family of membrane-bound proteins that function as intermediates in signal transduction pathways involved in cell growth and differentiation (91). N-ras provides a steady-state antiapoptotic signal, at least partially by regulating the duration of the jun-n terminal protein kinase activity triggered by an apoptotic challenge (92). Fibroblasts that lack N-ras expression are highly sensitive to the induction of apoptosis by variety of agents (92).

**NTT4 (=Rtx 1)** gene was upregulated after 8h waking and sleep deprivation (4).

NTT4 is an orphan member of the Na/Cl-dependent neurotransmitter transporter family, with presently unknown natural substrate. NTT4 is found in the CNS where it is exclusively distributed in neurons, highest levels are found in the cortex, hippocampus, thalamus and cerebellum. The immunoreactivity is found mostly in neuronal processes, where NTT4 appears to be a vesicular membrane bound protein, unlike the rest of the Na/Cl-dependent neurotransmitters. NTT4 is expressed mostly in glutamatergic terminals and in some subpopulations of GABAergic nerve endings (93).

**Protein tyrosine phoshpatase 1B (PTB1B**) gene was upregulated after 8h waking and sleep deprivation (4).

PTP1B is a widely expressed nontransmembrane protein tyrosine phosphatase, that can dephosphorylate several receptor tyrosine kinases. Most peptide growth factors, including insuline, signal through receptor tyrosine kinases. Their signalling capacity is determined by the balance between receptor tyrosine kinase activity and the activity of the tyrosine phosphatases. It has been shown

that PTP1B is involved in the insulin signalling pathway (94). One known signalling pathway is the insulin-like growth factor type I (IGF-1) receptor (IGF-IR), which PTP1B can regulate. The loss of PTP1B can enhance IGF-I -mediated cell survival, growth and motility (95)

**Rlf** gene was upregulated after 3h waking and sleep deprivation (4).

Ral guanide nucleotide exchange factor (RalGDS)-like factor (Rlf) is expressed ubiquitously and interacts with Ras: rlf mediates a distinct Ras-induced signalling pathway to gene induction. Ras is a small, ubiquitously expressed GTPase that regulates apoptosis and cell proliferation (96,97). Ras protein moderates cellular responses at several mitogens and/or differentiation factors and at external stimuli (97). Constitutively active form of Rlf can stimulate transcriptional activation and cell growth (98).

**12S rRNA** was upregulated after 3h waking and sleep deprivation (4).

12S rRNA is the small subunit of mitochondrial rRNA. It can be noted that mitochondria have their own DNA which directs mitochondrial protein synthesis.

**Serum/glucocorticoid-induced Serine/ threonine kinase (SKG1)** gene expression was increased after 8h waking and sleep deprivation (4).

SKG1 is activated *in vivo* in response to signals that activate phosphatidylinositol-3 kinase (PI3), e.g. by $H_2O_2$ and insuline growth factor (99). It has been suggested that SGK mediates the biological outputs of PI3 signalling, including cell survival and cell cycle progression (100).

**STAT 3** gene expression was increased after 8h waking and sleep deprivation (4).

STAT 3 is a member in a STAT (**s**ignal **t**ransducer and **a**ctivation of **t**ranscription) protein family, that is activated by cytokine receptors. Usually STATs are associated with transcriptional activation, often activating immediate early genes (101). While the rest of the family members are rather specific in their activation by a specific cytokine receptor, STAT3 is activated by a wide variety of cytokines, growth factors and other stimuli. In the brain STAT3 is specifically activated in response to ciliary neurotrophic factor (CNTF) and leptin. In acute phase response STAT3 appears to function as a classical transcription factor. Depending on the target tissue the outcome of STAT3 activation can be proliferation, cell survival or apoptosis (102).

**Synaptotagmin IV** gene expression was increased after 8 hours wake and sleep deprivation (4).

Synaptotagmin family consists of 11 members, thought to mediate synaptic function. Synaptotagmin IV is brain specific and is localized in the Golgi complex (103). It is induced in the hippocampus (104) and in cultured neurons (103) by depolarization. It has been suggested that induction of synaptotagmin IV is associated with development of the brain and in adults with synaptic plasticity (103).

**Thyroid hormone receptor Tr?** gene expression was upregulated after 8h waking and sleep deprivation (4).

The effects of the thyroid hormone $T_3$ are mediated through three receptors: $TR_1$, $TR_1$ and $TR_2$. Knockout mice that lack both 2 isoforms develop elevated concentrations of T4 and TSH, suggesting that this isoform is important in the negative feedback regulation of thryroid hormone levels (105). Thyroid hormone regulates energy metabolism and development.

**TIMP-1** gene expression increased after 8h waking and sleep deprivation (4).

Tissue inhibitor of metalloproteinase 1 (TIMP-1) is one of the four TIMPs that inhibit matrix metalloproteinases (MMP) (zinc endopeptidases), which play a role in tissue morphogenesis and would healing (106). The proteolytic activity of MMP:s is involved in extracellular matrix degradation, and is controlled by the TIMPs. The MMP/TIMP interaction has been implicated in several pathological processes, especially the formation of cancer metastasis, atherosclerosis and decreased fertility (107). In the brain both ischaemia (108) and neuronal excitation (109) induce the expression of TIMP-1.

Whether the increase in gene expression during sleep deprivation is increased because of increased neuronal activity or because of increased need to protect against proteolysis remains speculative.

The expression of **tissue plasminogen activator (tPA)** was increased in the cortex after 8h waking and sleep deprivation, while the expression of MMP-9 (matrix metalloproteinase-9) was decreased (18).

Tissue plasminogen activator belongs to a family of serine proteases, which exert a variety of functions in the body from food digestion to modification of extracellular matrix (110). tPA is broadly distributed in the brain, and the tPA produced by limbic neurons is secreted upon depolarization. It has been implicated in long-term potentiation, learning and memory, as well as in excitotoxic neuronal cell death (111).

**TrkB receptor** gene expression increased after 8h waking and sleep deprivation (4).

TrkB receptor belongs to a neurotrophin receptor family trk with three characterized members. TrkB receptor is specific for the brain-derived neurotrophic factor (BDNF) and neurotrofin 4/5. Neurotrophins mediate their actions through several signalling pathways, and depending on which signalling pathway is activated, neurotrophins may lead to cell survival, growth and differentiation or cell death(82). In addition to neuronal survival and differentiation, BDNF/TrkB has a role in regulation of

synaptic strength, suggesting a role in learning and memory processing (112).

**Tyrosine hydroxylase (TH)** is the rate limiting enzyme of the noradenaline synthesis. A 3-5 day REM sleep deprivation increased the amount of TH mRNA in the locus coeruleus but a shorter, 24h REM sleep deprivation, or 2-12 h total sleep deprivation were ineffective (90,113). Increase in the mRNA of the rate limiting enzyme can be regarded as a sign of increased need to synthetise the enzyme, and the end product, noradrenaline, which is released during the prolonged wakefulness period.

**Vgf** gene expression increased after 8h waking and sleep deprivation (4).

Neurotrophin responsive gene vgf is widely expressed in both central and periferial neurons and in some neuroendocrine cell populations (e.g.pituitary gonadotropes). The main activators of vgf are BDNF and NT3. Neuronal depolarization, as well as fasting can activate vgf expression(114,115). VGF knock out mice show alterations in their energy metabolism with hyperactivity and persistent hypermetabolic state, with markedly decreased levels of leptin and fat stores (115-117).

It is interesting to note that vgf mRNA levels are increased as response to light stimulation in the suprachiasmatic nucleus of hamsters at time points when light exposure can induce a phase shift of the circadian rhythm (118).

### 6.4. Sleep genes

Only few genes are inducted by sleep. c-fos gene expression is increased during sleep in the ventrolater preoptic area (VLPO) of the hypothalamus, presumably indicating increased cellular activity in this area. The cells which express c-fos during sleep are concentrated in a small area, and send projections to waking promoting nuclei, including the histaminergic nuclei (119). The cells are GABAergic, and most of them contain also galanin (119). It has been suggested that these cells play a central role in the initiation and maintenance of sleep (1,120). However, the target gene of the increased c-fos production is unknown.

Recently a group of genes has been identified that are expressed more during sleep than waking: RNA binding proteins U4, RBM3 and Cirp (121).

**Cirp and RBM3** are members of the glycine rich RNA-binding protein (GRP) family. They are cold-inducible: a mild hypothermia of 32 C induces both proteins (122,123). Cirp expression displays diurnal variation in the suprachiasmatic nucleus and in the cortex of mice, but the variation discontinues in constant darkness (124). It has been suggested that Cirp acts as a RNA chaperones (122).

**U4** is one of the small nuclear ribonucleoproteins (snRNP) that in the course of pre-mRNA maturation form the spliceosome, which is needed for the splicing of the mature mRNA from the pre-mRNA. Thus increase in U4 mRNA suggests increased mRNA processing, and possibly increased protein synthesis.

The expression of the gene coding for the enzyme **choline acetyltransferase** in the rat vertical and horizontal limbs of Broca was higher during the day than night, and low during wakefulness, intermediate during slow-wave sleep and high during REM sleep (6).

## 7. CONCLUSIONS

A multitude of genes that are activated either during wakefulness/prolonged wakefulness or sleep have been characterized. A vast majority has been recognized using cDNA arrays (21). One intriguing feature is that different research groups have rarely found the same genes to be activated: e.g. cDNA arrays have not shown increases in tyrosine hydroxylase or GHRH gene expression. Taking in consideration the different tissues, different periods of wakefulness and different analysis methods this is hardly surprising, but nevertheless, unsatisfactory.

The genes activated during wakefulness/ prolonged wakefulness can be grouped according to the known or hypothesised function(s) of the genes. It should be noted that the function of many genes are presently incompletely understood. However, some ideas may arise from the genes whose function already are fairly well characterized.

Prolonged wakefulness is associated with increased neuronal activation with increased demand of release and uptake of the neurotransmitters. Usually the extracellular concentrations of neurotransmitters are higher during waking and prolonged wakefulness than sleep (17,125,126) reflecting the higher activity of neurons during waking. Naturally this also means higher energy consumption during wakefulness than sleep (127-129). As calcium is one of the most common regulators of signal transduction, molecules that are involved in regulation of $Ca^{2+}$ concentrations are also expected to be affected by vigilance states. The transcription and translation of proteins share common organelles and also molecular components (e.g. chaperons). It is to be expected that the synthesis of these components is activated upon increased protein synthesis. Genes belonging to each of these categories have been found to be activated after a period of prolonged wakefulness (see above). A reasonable hypothesis is, that the need of neurotransmitters, transporters and molecules of energy metabolism could lead to increased synthesis of these substances, preceded by increased mRNA levels. This would represent a natural response of the body to the waking state, and explain the majority of the increased gene expression.

The neuronal activity during waking precedes sleep. It has been suggested that sleep is needed to compensate the consequences (e.g. energy consumption) of the neuronal activity. Thus activity during waking could produce substances that create sleep pressure and induce sleep. Whether any of the molecules that so far have been

found to be increased during waking contribute to the increased sleep propensity remains to be established.

The flow of information from extracellular sources into the cell and inside the cell to the nucleus to regulate transcription and translation is continuous during wakefulness. Signal transduction pathways are activated, and the signal is transmitted through complicated intracellular networks. Whether the signal will activate a protein synthesis depends on several factors, e.g. convergence from other signalling pathways. The knowledge of these processes is still incomplete, but new information is accumulating fast. It is out of the scope of this presentation to extensively review the signalling molecules and pathways whose genes have been found activated during waking/prolonged wakefulness. A short summary of each molecule is provided, but for a deeper understanding further reading of more specific topis is recommended.

In addition to these gene categories, other types of genes are activated during prolonged wakefulness: genes, that prepare the organism to prevent destruction, like the heat shock proteins, molecules involved in both initiation and prevention of cell destruction via apoptosis (e.g. CHOP, Metallothionein 3, STAT and N-ras), and very interestingly, a lot of molecules that are involved in neural plasticity (e.g. Arc, dendrin, NGFI-A and tPA).

Presently it is too early to make conclusions concerning the purpose of sleep based on these findings, but certainly, the findings give rise for interesting speculations. For instance: is it a coincidence that warm-induced genes (heat shock proteins) are wake-active, while cold induced genes are sleep-active?

## 8. PERSPECTIVE

Measurement of vigilance state-associated gene expression is a fairly novel technique in sleep research. Regardless, a wealth of novel information has already been gathered. As the techniques, as well as the experimental designs have been different in different laboratories, uniform results have rarely been obtained.

The extraction of novel genes has created a lot of new ideas, and will direct future experiments, that use complementary techniques. It is possible that completely new avenues will be opened for research, based on the cDNA arrays, as genes that were not expected to be associated with vigilance state regulation will be found. An example of this is the discovery of the connection of the orexin/hypocretin gene to narcolepsy.

A more complete understanding of intracellular signalling, mechanisms of neural plasticity, learning and memory, as well as understanding of the role of heat shock proteins and apoptosis in normal brain function will help to clarify the role of the presently known vigilance state-associated molecules of sleep regulation - and maybe help to solve the question why we have to sleep.

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