Chapter 7: Discussion

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Discussion

Yoga, an ancient Indian science and way of life has gained extensive attention from the scientific community world over with the focus on understanding the application of yoga as a mind-body intervention for clinical conditions. Earlier studies have shown Yoga practices to decrease the cholesterol levels, blood pressure and promote cardio-protective activity (Gokal, Shillito, & Maharaj, 2007; Jayasinghe, 2004; Sinha, Singh, Monga, & Ray, 2007) recognizing yoga as a non-pharmacological intervention in the management of hypertension (Cramer et al., 2014) . Also, yoga practices have been shown to decrease the insulin resistance and promote overall quality of life in diabetes patients (Chaya et al., 2008). In healthy individuals, yoga practices promote wellness and performance (Dash & Telles, 2001; Subramanya & Telles, 2009). However, not many studies are presently available to describe the molecular basis of Yoga practices. An earlier study by Qu et al., has shown rapid gene expression changes following a yoga intervention of only 2 hours (Qu, Olafsrud, Meza-Zepeda, & Saatcioglu, 2013). Qu et. al., indicated that there were more differentially expressed genes following yoga practices compared to a control session involving listening to music and no specific pathways were significantly differentially regulated following yoga intervention (Qu et al., 2013). Another study with meditation on dementia care givers showed repression of NFκB associated transcription of pro-inflammatory cytokines (Black et al., 2013). A recent review on various mind-body interventions concluded that practitioners of mind-body intervention techniques might decrease the risk of inflammation related diseases (Buric, Farias, Jong, Mee, & Brazil, 2017). In the current study, the effect of Yoga was assayed for changes at biochemical, psychophysiological and gene expression level by comparing with control group. With an 87

day intervention this study reports the results of longest Yoga intervention thus far. Also this is the first study developed to understand the mechanisms of action of Yoga practices on facilitating adaptation under extremely stressful conditions. Earlier studies in normal environmental conditions have proven Yoga as an effective tool for non-pharmacological management of non-communicable diseases and overall well-being. Here the role of Yoga practices in facilitating '*optimal and efficient adaptation*' in Antarctica is unraveled. In addition, this study has provided preliminary directions on targets and probable mechanisms of action of Yoga practice in various non-communicable diseases.

Sample/data collection was done at four time points. Psychological assessments were made at baseline (BL) and on departing from Antarctica (P3). The biochemical assessments and Chemiluminescence assays were made on samples obtained on reaching Antarctica (P1), departing from the first Indian station (P2) and on departing from Antarctica (P3). Single colour global Gene Expression analysis were performed on samples obtained at baseline (BL), on reaching Antarctica (P1), and on departing from Antarctica (P3).

The results from our study suggest a contrasting 44.4% increase and 32.84% decrease in the subjective sleep quality in the yoga and control group respectively. Daytime dysfunction was the most influenced component by Yoga practices at Antarctica. Similarly, a significant 33.56% reduction in Perseverative thinking was observed in Yoga group whereas, in control group 22.88% increase was noted. The biochemistry tests demonstrated significant increase in Triglyceride, LDL, HDL and total cholesterol levels between P1 and P2 in the yoga group. In the control group, a consistent non-significant increase was noted in the lipid profile between P1, P2 and P3. In yoga group, elevated lipid profile is also accompanied by increased gene expression of Endothelin Receptor Type A (*EDNRA*). EDNRA, a G protein-coupled receptor,

when bound to Endothelin results in prolonged vaso-constriction and decrease in lipid binding to the vasculature(Ballinger, Ivey, Osman, Thomas, & Little, 2009) promoting cardio-protective and anti-atherosclerotic effect associated with elevated lipid profile. Also, Genes associated with lipolysis and glycogenolysis were upregulated in the initial phase of expedition BL-P1. From the changes in the lipid profile, we speculate that, upregulation of scavenger receptor *CD36* and *MAPK1* in control group (from gene expression data) is associated with increase in oxidised/dysfunctional HDL (Sini, Deepa, Harikrishnan, & Jayakumari, 2017). On the contrary repression of *MMP9* in the yoga group demonstrates the cardio-protective role through reduction of oxidative stress. These changes indicate that Yoga might facilitate adaptation by promoting insulation to decrease heat loss. Interestingly γ- glutamyl transferase (GGT), an enzyme involved in detoxification, decreased from P1 to P2 and increased back to the levels of P1 at P3 in both the groups indicating the hepato-biliary system was under stress in extreme environmental condition. However, the contributory role of alcohol intake on GGT 36 hours before the blood collection requires to be addressed(McPherson & Pincus, 2017).

Throughout the three timepoints, RNA binding and nucleic acid binding genes were upregulated in both the groups. RNA binding proteins (RBP) regulate stability, maturation and turnover of all RNAs. Dysregulation of RBPs are associated with developmental and neurological disorders (Gerstberger, Hafner, Ascano, & Tuschl, 2014). In the Control group upregulation of ribosomal protein production was seen consistently in all the timepoints. Increased expression of ribosomal proteins suggests its role in extra-ribosomal functions such as replication, transcription and even aging apart from the regular functioning of RNA synthesis (Bhavsar, Makley, & Tsonis, 2010). Increased transferase activity was observed in

both the groups at BL-P1 throughout the expedition might be required for prevention of alzheimer's and huntington's disease(Oda, 1999; Smith et al., 2006).

Interestingly, in BL-P3 timepoint comparison for Yoga group, no ontology classes for molecular function and biological process were significantly overrepresented. Whereas, in the control group, genes associated with post-transcriptional modification and translation were overrepresented. Increased translation might be responsible for more number of differentially regulated genes in the control group than the yoga group. Over-representation of genes in BL-P3 of control group associated with process regulating structural integrity of ribosomes, enzyme regulation, metabolic functions of cell growth, metabolism of nitrogen compounds, nucleobase containing compound process and cellular physiological process indicates increased cell proliferation and replication.

Throughout all the timepoints, both the Yoga and Control groups showed repression of sensory perception, sensory perception of chemical stimulus, smell and neurological system process. Perception of smell and taste has been found to be less appreciated in cold temperatures (Institute of Medicine Committee on Military Nutrition Research of the USA, 1993). In the control group, the underrepresentation of the above process might be through repression of G Protein Coupled Receptors [*GPCR*]. *GPCR* has wide a wide ligand range from peptide hormones, neurotransmitters to odour molecules and is the largest family of receptors with involvement in many different functions. To name a few, it is associated with behaviour, mood regulation (Catapano & Manji, 2007; Huang & Thathiah, 2015); regulation of immune system activity and inflammation (Sun & Ye, 2012), regulation of autonomic nervous system(Billington & Penn, 2003; Mighiu & Heximer, 2012) , growth and metastasis of tumors (Dorsam & Gutkind, 2007) and homeostasis modulation (Hazell et al., 2012; Thorens, 2010).

For the entire expedition (BL-P3), the homeobox and helix-turn-helix transcription factors were repressed in the control group. The repression of homeobox genes might be partially attributed to the altered androgen levels in Antarctica (Daftary & Taylor, 2006; Sawhney et al., 1998) and might be associated with increased apoptosis and malignancy (Shah & Sukumar, 2010). Homeodomain transcription factors also have wide area of influence in terms of the expression of the genes they regulate. Transcription factors with homeodomain control processes like pluripotency, cellular differentiation and are critical during embryogenesis. One of the significant findings of the study is the identification of the GPCR and homeobox class of transcription factors, which by the virtue of their global influence should help in better understanding of the effect of extreme climatic conditions on physiology.

Interestingly in the initial phases of expedition during sea voyage, in the Yoga group only the most vital physiological processes – metabolic and cellular processes were upregulated, whereas, in the control group, apart from the above essential processes, transcription, translation, cellular component biogenesis and associated phosphate metabolism were upregulated throughout the expedition. These indicate the differences in the approach in regulating adaptation in the Yoga and control groups: while in Yoga group the emphasis is on cell survival, in the control group the mechanism seems to be moving towards cell proliferation. Also in the control group apoptosis function was seen to be induced, therefore prompting to speculate whether the increase in cell proliferation is an adaptation to combat apoptosis. These observations from our data direct us to suggest that Yoga might facilitate optimal adaptation.

To understand this phenomena we performed a stringent analysis to list the DEGs from the genes that were present in all the 12 samples across three timepoints. The rationale for

performing this list was to identify those genes that are commonly regulated irrespective of an individual's inherent genetic constitution. Genes associated with Zinc finger transcription factors were over-represented in Yoga group during both the initial and later phases of the expedition. The purpose and their implication in regulating the physiology needs to be known. There were no processes that were significantly over or under-represented in BL-P3 timepoint comparison of both yoga and control groups. It is interesting to note that in earlier two studies on yoga by Qu et.al., and Saatcioglu no specific group of genes were regulated and the genes regulated were from diverse categories (Qu et al., 2013; Saatcioglu, 2013). We speculate that the reason for no distinct class or group of genes to be differentially regulated might be because of the reason that every individual in the group tries to adapt to the stressful environment depending on the strengths and weaknesses of the individual's inherent genetic makeup.

Earlier, in the routine analysis of differentially expressed genes, no group of genes of molecular function or biological process were over represented for the condition BL-P3. We speculate that it might possibly be because the adaptation might have happened and the most efficient regulation required might have already been adopted.

Hence, from the above analyses, in line with a well-established fact our data from the control group suggests that the body is capable of adapting to the environment by regulating multiple processes which the system perceives to be the best for that situation. However, the benefit of the regulation adapted in the long run needs to be ascertained. With yoga as intervention, the regulation becomes optimal and efficient and appears to be logically appropriate for the environment. But, how yoga is capable of identifying and regulating the most appropriate process despite being in a novel environment needs further exploration.

In the section below, we intend to explicitly explain how Yoga facilities efficient and optimal regulation through explaining physiological scenarios through pathways that were significantly regulated in the Yoga and Control groups. Few physiological scenarios are presented as pathways to support our hypothesis.

1. Cellular response to Stress:

The cell's response to a stressful condition decides its fate. A cell in response to an adverse environment might adapt or end up in apoptosis. Stringent regulatory measures are required for adaptation especially on key signalling molecules of inflammation and oxidative stress to prevent triggering any alarming signals that might hinder the process of adaptation. Simultaneously, checkpoints should be enabled to ensure eventual increase in apoptosis not to adversely affect cell cycle regulation. This entire process requires appropriate contributions from multiple systems: metabolism, cell cycle, immunity and salvage pathways.

The processes associated with gene silencing were upregulated in both yoga and control group throughout the entire phase of expedition. To facilitate gene silencing, RNA Induced Silencing Complex [*RISC*] that block the initiation of translation (Podshivalova & Salomon, 2013; Pratt & MacRae, 2009) were upregulated in the control group. Interestingly, in the control group, histone genes associated with epigenetic regulation were overexpressed suggesting epigenetic involvement in the control group.

During states of stress at the cellular level, immune system and intracellular signalling pathways interact with each other to manifest response. Toll like receptors (*TLR*) are pattern recognition receptors that detect the pathogen associated molecular pattern leading to innate immune response. The TLRs also respond to damage associated molecular pattern signals

derived from damaged cells in a stressful environment. *TOLLIP*, an evolution conserved endogenous regulator of Toll signalling pathway when over-expressed, inhibits *TLR2* and *TLR 4* modulating immune response (Luiz, Santos Júnior, Bonetti, Brandeburgo, & Yen, 2014) by forming complexes with IRAK (interleukin-1 receptor associated kinase) family(Akira & Takeda, 2004). IRAK is a kinase which has been shown to upregulate *NFκB* (nuclear factor kappa-light-chain-enhancer of activated B cells). It is shown that upregulated *TRAF6* subsequently degrades *IRAK1*, thereby influencing activation of *NFκB*(Liu, Park, & Abraham, 2008). Whereas, another subfamily of IRAK, *IRAK4* when upregulated, cannot effectively activate *NFκB* like *IRAK1* (Akira & Takeda, 2004).

From our results it appears that the upregulated TLRs in both the groups facilitate stress response. In the control group, the stress response is regulated by *TRAF6,* which degrades *IRAK1-TOLLIP* complex, facilitating NFκB production. Whereas, the upregulated *IRAK4* in the Yoga group cannot effectively activate NFκB production. The upregulated genes associated with superoxide production in the control group might be due to increased metabolism.

Heightened cortisol levels are known to activate TLR signalling pathway (Lancaster et al., 2005). As a response to increased cortisol levels in both the groups, the *TRIF* dependant toll like receptor signalling pathway might have been activated to counter any incoming pathogenic stimuli. While there was an inflammatory response noted in the Control group mediated by the production of *NFκB,* the Yoga group had an upregulated MyD88 independent TLR pathway. MyD88 independent TLR pathway when activated is known to produce an immune response without triggering genes encoding pro-inflammatory cytokines (Akira & Takeda, 2004; Kawai et al., 2001).

Telomere maintenance genes were downregulated in both the groups. Both Yoga and Control groups had upregulated DNA confirmation change and chromatin assembly. DNA confirmation change and better chromatin assembly are essential to minimise the DNA damage and control gene expression and replication(Ridgway & Almouzni, 2001). DNA conformational change is a process that are understood to play vital role in forming framework for long term epigenetic regulation and maintenance of genome(Sims, Nishioka, & Reinberg, 2003). In the Yoga group genes associated with histone modification and histone lysine methylation were downregulated – probably as a means to prevent any impact of the adverse stressor on the epigenetic memory.

Genes associated with response to temperature were downregulated in the Yoga group whereas no significant regulation was observed in the control group. Upregulated ubiquitination mediates membrane protein trafickking and degradation of cell (d'Azzo, Bongiovanni, & Nastasi, 2005; Teixeira & Reed, 2013). Protein catabolism mediated by ubiquitination and its feedback regulatory genes associated with negative regulation of ubiquitin protein ligase activity were upregulated in the control group suggesting an increased degradation of cells in response to stress.

Oxidative stress induces apoptosis (Buttke & Sandstrom, 1994). Cell survival was promoted in the Yoga group by upregulating the genes that negatively regulate cellular response to oxidative stress and oxidative stress induced cell death and repressing signal transduction through p53 mediator. On the contrary the same responses were downregulated in the control group suggesting increased oxidative stress and cell death. In response to increased oxidative stress induced DNA damage in the control group, genes associated with nucleotide excision repair, interstrand cross link repair and DNA integrity checkpoint were upregulated. Previous

studies in our laboratory (unpublished) have shown reduction in DNA damage following yoga practices. We attribute two reasons that might have resulted this observation in Yoga group (i) the DNA damage per se could have reduced (ii) as an attempt to promote cell survival and reduce apoptosis while maintaining the genome integrity.

In the Yoga group an increase in mitochondrial membrane permeability is observed. Several stressors like pH>7, pyridine nucleotide oxidation, ROS formation and increased mitochondrial $Ca2^{+}$ are all factors that act synergistically increase mitochondrial membrane permeability *in-situ* in living cells. However, other factors like glutathione oxidation, mitochondrial depolarization, increased intracellular P_i and accumulation lysophosphatides and free fatty acids are also associated with increased mitochondrial membrane permeability(Lemasters et al., 1998). We speculate that this increase in mitochondrial membrane permeability in Yoga group is associated with the increased free fatty acids and depleted glutathione peroxidase. Acyl CoA, a group of co-enzymes, when bound to the end of long chain free fatty acids undergoes beta-oxidation to form one or more molecules of acetyl-CoA, which later enters inside Citric acid cycle eventually forming ATP. The fatty acids in the form of acyl CoA are known to be excellent respiratory substrates for mitochondria of most tissues. Their oxidation is coupled to the generation of high energy state of the mitochondrial membrane and, consequently, to ATP synthesis. Simultaneously, the lipid biosynthetic processes and the cellular response to reactive oxygen species were downregulated.

Nuclear pore complex is a multifaceted and intricate protein complex that is now recently being understood to play critical roles in regulation of gene expression(Texari et al., 2013; Van de Vosse et al., 2013). The role of nuclear pore complex have also been implicated in diverse human pathologies including auto-immune diseases, infections and cancer (Hatch &

Hetzer, 2014; D. N. Simon & Rout, 2014) apart from well understood roles of nucleocytoplasmic transport(Kabachinski & Schwartz, 2015). Results from our study indicate downregulation of Nuclear pore organisation in the Yoga group and upregulation of the same in the control group to regulate the transfer of proteins and RNA between the Cytoplasm and nucleoplasm.

Similar to a recent finding on relaxation response(Bhasin et al., 2013), in the yoga group, the genes associated with cellular response to insulin stimulus were downreglated, which downstream, represses genes associated with negative regulation of TOR. Whereas, no regulation regarding insulin was observed in the control group and TOR signalling was upregulated.

Similar to the previous observation in Antarctic stations, our data shows upregulation of genes associated with shedding of latent virus by formation of inclusion body assemblies (Mehta, Pierson, Cooley, Dubow, & Lugg, 2000) in both the groups.

In the latter half of the expedition, the cellular response to stress had a different signature in both the groups. Genes associated with gene silencing, nuclear pore complex assembly, signal transduction by p53, telomere capping and organisation were upregulated in both the groups. In the Yoga group, the genes associated with Glycogen biosynthesis, regulation of mitochondrial membrane permeability, apoptotic nuclear fragmentation and DNA catabolic process involved in apoptosis and nucleotide excision repair were downregulated. The repression of mitochondrial membrane permeability might be an attempt to prevent apoptosis. The TLRs, *TLR2-6* were downregulated. Overexpressed *TLR1* resulted in PI3K-Akt signaling pathway mediated pro-inflammatory cytokine production in the Control group. Caspase mediated apoptotic pathway was triggered in the control group. *TAB2* and *TAK1* mediated

activation of *NFκB* were observed in both groups. Overexpression of *TOLLIP* in the later phase of the expedition might inhibit *TLR2* and *TLR4* to decrease produciton of NFκB.

In the Yoga group, genes associated with positive regulation of DNA double strand breaks, interstrand cross link repair, homologous recombination were upregulated. Genes associated with chromosome integration which are responsible for genome integrity and cellular response to temperature were upregulated. As a contrast, in the control group, the genes associated with DNA damage response and DNA integrity checkpoint were downregulated. Genes associated with mitotic metaphase/anaphase transition of cell cycle indicating frequency of cell cycle were upregulated. Interestingly genes associated with chromatin assembly were downregulated, probably making the genetic material more susceptible to damage and increased transcription – which was corroborated by increased histone acetylation. Genes with antioxidant property were upregulated to remove superoxide radicals. Genes associated with Mitochondrial autophagy were upregulated. Glutathione peroxidase activity was downregulated probably due to depletion of the glutathione entities. Genes associated with Error free translesion synthesis were upregulated as a measure to promote genome integrity.

Results from the microarray suggest that on exposure to stressful environment during sea voyage, selenocompound and seleno amino acid metabolism were up-regulated in both Yoga and Control groups suggesting the body's efforts to minimise inflammation and prevent cancer(Brown & Arthur, 2001; Moghadaszadeh & Beggs, 2006).

From the data it appears that in the initial phase of the expedition, the Yoga group had signals promoting cell survival by decreasing activation of NFκB pathway and upregulating negative response to oxidative stress and increasing mitochondrial membrane permeability to facilitate energy production. Also, yoga group was able to elicit an immune response through non-

inflammatory MyD88 independent TLR pathway. In the later phase of the expedition, the yoga group appeared to have upregulated genes to perform DNA repair and decrease oxidative stress and promote cell survival by decreasing mitochondrial membrane permeability. Whereas, in the Control group, the data suggest that throughout the expedition, there was upregulated NFκB activity, and caspase mediated apoptosis. Interestingly, the despite having upregulated DNA repair mechanisms, the upregulated chromatin assembly genes responsible for maintaining the integrity of the genetic material was downregulated, making the cell more susceptible to damage.

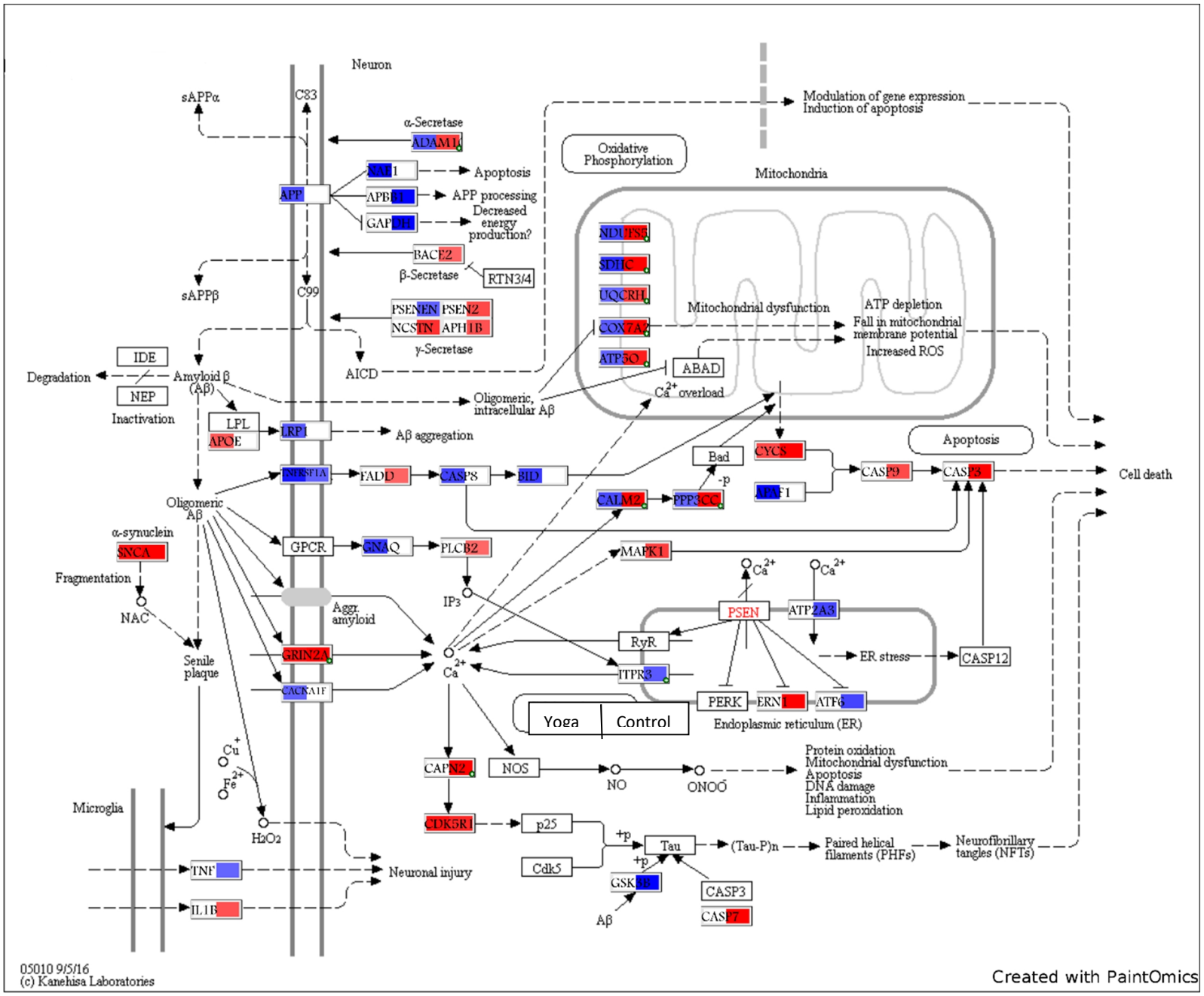
2. Cell Survival & Apoptosis

Comparison was made between the groups for the entire expedition duration [BL-P3] to understand the regulation of cell survival and apoptosis.

Amyloid Precursor protein (*APP*), which when cleaved through the β and γ secretase pathways results in β-amyloids; while the α-secretase pathway is non-amyloidogenic. Interestingly, *APP* was repressed in the Yoga group. Whereas, the genes associated with amyloidogenic β and γ secretase pathways were upregulated in the Control group. As a feedback regulation, upregulated *ADAM17* in Yoga group, promotes production of neuroprotective soluble *APPα* fragments and reduces amyloid β generation(Qian, Shen, & Wang, 2016). *NAE1/APPBP1*, which interacts with APP and results in apoptosis(Chen et al., 2014) was downregulated in Yoga group which might indicate better neuronal protection. Downregulation of *APPBP1* also indicates a probable regulation of Yoga in delaying cell-cycle progression in the S-M checkpoint(Chen et al., 2014).

Yoga group had selectively downregulated apoptosis inducing *CASP8, BCL-2* and *BID* preventing the downstream components of mitochondria from inducing apoptosis(Li, Zhu, Xu, & Yuan, 1998; Luo, Budihardjo, Zou, Slaughter, & Wang, 1998).

In the control group, Amyloidogenic pathway through the involvement of β-secretase and γ-secretase pathways is observed.


Neuron
sAPPα
C83
α-Secretase
ADAM10
Modulation of gene expression
Induction of apoptosis
Oxidative Phosphorylation
Mitochondria
APP
Apoptosis
APP processing
Decreased energy production?
BACE2
β-Secretase
RTN3/4
sAPPβ
C99
PSENEN PSEN2
NCSTN APH1B
γ-Secretase
AICD
NDUFS3
SDHC
UQCRH
COX7A2
ATP5O
Mitochondrial dysfunction
ATP depletion
Fall in mitochondrial membrane potential
Increased ROS
ABAD
Ca2+ overload
IDE
Degradation
NEP
Inactivation
Amyloid β (Aβ)
Oligomeric, intracellular Aβ
LPL
APOE
LRP1
Aβ aggregation
TNFRSF1A
FADD
CASP8
BID
Bad
-p
CYCS
APAF1
CASP9
CASP3
Apoptosis
Cell death
CALM2
PPP3CC
Oligomeric Aβ
α-synuclein
SNCA
Fragmentation
NAC
GPCR
GNAQ
PLCB2
MAPK1
Aggr. amyloid
IP3
Ca2+
PSEN
ATP2A3
RyR
ITPR3
ER stress
CASP12
Senile plaque
GRIN2A
CACNA1F
PERK
ERN1
ATF6
Yoga
Control
Endoplasmic reticulum (ER)
Cu+
Fe2+
CAPN2
NOS
NO
ONOO-
Protein oxidation
Mitochondrial dysfunction
Apoptosis
DNA damage
Inflammation
Lipid peroxidation
Microglia
H2O2
CDK5R1
p25
+p
Tau
(Tau-P)n
Paired helical filaments (PHFs)
Neurofibrillary tangles (NFTs)
TNF
Neuronal injury
Cdk5
+p
GSK3B
CASP3
IL1B
Aβ
CASP7
05010 9/5/16
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3. DNA Replication:

DNA must be replicated with high fidelity to ensure genome integrity. Simultaneously during the process of replication, DNA damage must be effectively repaired to prevent any mutations or oncogenic response, whilst the DNA is involved in other processes like recombination and transcription..

Replication starts at the the origin sites of replication marked by the formation of a pre-replicative complex (pRC) in the G1 phase of Cell cycle by assembly of origin recognition complex (ORC1 to ORC6) and additional replication factors, cell division control protein 6 (cdc6), chromatin licensing and DNA replication factor 1 (Cdt1) and the mini chromosome maintenance (MCM) helicase. Pre-replicative complex starts with the recruitment of ORC, which forms the foundation, followed by cdc6, cdt1 and MCM complex. This is followed by initiation of replication by DNA polymerase α (polα) having four subunits, two regulatory subunits POLA1 and POLA2 and two primases PRIM1 and PRIM2. At each fired origin site, two replication forks are established that move in the opposite direction – along the leading and lagging strands – and move away from the origin as the helicase unzips the parental DNA. Two polymerases: Polδ replicates both leading and lagging strand while Polε replicates the lagging strand (Johnson, Klassen, Prakash, & Prakash, 2015).

Our data indicates a contrasting regulation of the replication pathway between the groups. All the four subunits of Polα: POLA1, POLA2, PRIM1 and PRIM2 were downregulated in the Yoga group but were upregulated in the control group indicating a repressed replication in the Yoga group and an increased replication in the control group. Also, MCM 2-7 were downregulated in the Yoga group. MCM complex is mainly involved in helicase activity (unwinding the DNA) both during initiation and elongation phases of DNA replication. In the

control group Polδ, the major replicase was downregulated and Polε that replicates the lagging strand was upregulated. Observations from model organisms indicating that Polε as not essential for replication(Suyari et al., 2012), whereas, Polδ as indispensable for viability of cell (Boulet, Simon, Faye, Bauer, & Burgers, 1989; M. Simon, Giot, & Faye, 1991) supports the role of Polδ as the major replicase. Apart from the replication, both Polδ and Polε are involved in multiple DNA repair mechanisms and interestingly, they substitute for one another in nucleotide excision repair(Loeb & Monnat, 2008). Interestingly among the six ORCs, only ORC2 was upregulated in the control group and other, ORC3-6, were all downregulated. In a study Stoeber et al., have demonstrated that ORC2-5 is present throughout the proliferative cycle without necessarily meaning that the cells are in active replication cycle (Stoeber et al., 2001), in other words, they are not cell cycle regulated. The same study also shows that ORC6 is involved in recruiting Cdc6, which is one of the licensing proteins along with MCM.

The replication starts in the presence of other replication factors including human replication protein A (RPA), the clamp loader (RF-C) and the clamp (PCNA). The process of replication can be hampered by any impeding such as a protein DNA complex or DNA damage. Multiple checkpoints ensure progression of the replication forks. When one replication fork is terminally blocked or arrested, the adjacent origin site is fired to inhibit any further replication. (Laskey & Harland, 1981; Zegerman & Diffley, 2009). Human replication protein A (RPA) is a heterodimeric protein complex, whose role is to prevent unwound DNA from winding back on itself (Flynn & Zou, 2010), is involved in replication, repair, cell cycle and DNA damage checkpoints. It gets hyper-phosphorylated in response to the DNA damage events or replication stress by checkpoint kinases including ataxia telangiectasia mutated (ATM), ATM and Rad-3 related (ATR) and DNA dependant protein kinase (DNA-PK)(Zou, Liu, Wu, & Shell, 2006).

Unlike the other RPA subunits that facilitate replication, RPA4, a recently characterised subunit, when expressed leads to cell cycle arrest at G2/M phase. In addition, RPA4 localized to the sites of DNA damage and reduced γ-H2AX caused by RPA-2 depletion supporting maintenance of genomic integrity of a cell(Haring et al., 2010). Proteins RF-C and PCNA are involved in forming the scaffolding required to assemble and hold different proteins/enzymes required for DNA based processes like replication, recombination, chromatin remodeling etc. RFC couples energy from ATP hydrolysis to open and close the circular PCNA over the primed sites on DNA which will be used by polymerases.

A subunit of RPA, RPA1 facilitating replication was upregulated in control group. Interestingly, in both the groups, RPA4 was upregulated. Other replication factors the clamp loaders RFC1, RFC4 and the clamp PCNA were downregulated in Yoga group. Whereas, the clamp loaders RFC 4, RFC 5 were upregulated and the clamp PCNA was downregulated in both groups. The DNA2 helicase interacts with RPA to process the double strand breaks, okazaki fragments and stalled replication forks to maintain genome integrity.

DNA replication and cell cycle are closely associated. The Eukaryotic cell cycle is controlled by a critical regulatory network which is conserved from yeast to humans. Cell cycle in eukaryotes is grouped into three waves: (i) G1-S phase (ii) G2 to M phase and (iii) M to G1 phase(Bähler, 2005). In humans, the M to G1 phase transition is not clearly defined(Fukuoka et al., 2013). DNA structure checkpoint, which arrest the cell cycle in response to DNA damage or incomplete replication and the ‘restriction point’, the point in cell cycle beyond which the cell becomes committed to enter and progress the cell cycle independent of the signals from the environment – act as two crucial aspects of cell cycle regulation. In human cells, the G1-S transcription depends on the E2F family of transcription factors and their dimerization partner

proteins. E2F family members are associated with transcriptional activation (E2F1, E2F2 and E2F3A) or repression (E2F3B, E2F4, E2F5, E2F6, E2F7 and E2F8). Recent evidence also suggests the role of activator E2F to act as repressors and vice versa. In addition to the E2F proteins, including RB, p107 and p130, that bind and inhibit the expression of E2F regulated genes(Helin, 1998).

Our results considering the entire expedition [BL-P3] suggest that, the cell cycle was facilitated in the control group whereas, checkpoints were present in Yoga group to delay the cell cycle. The transcriptional activators E2F2 and E2F3 were overexpressed in the control group. DNA damage checkpoint ATM and ATR were both upregulated in the control group indicating a possible negative regulatory mechanism to inhibit cell cycle proliferation and facilitate p53 mediated apoptosis. The cells with minimal stress might have low or minimal ATM levels and presence of ATM is not critical for normal cell cycle progression or cellular differentiation(Shiloh & Kastan, 2001).

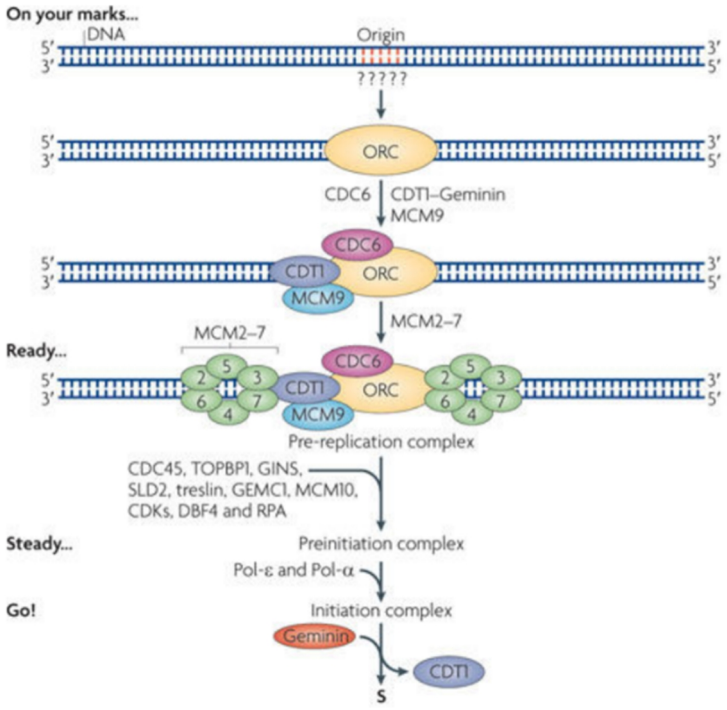
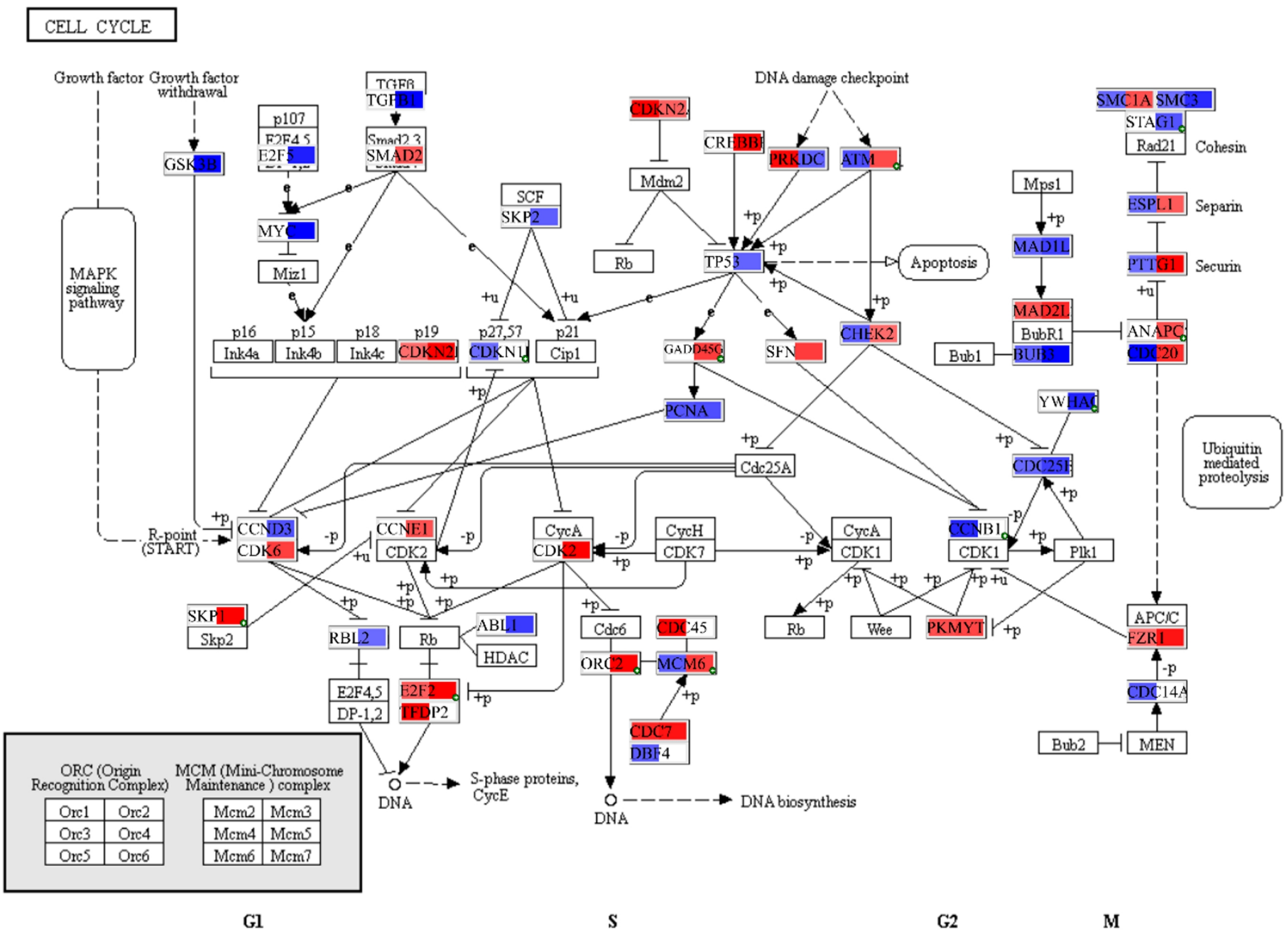
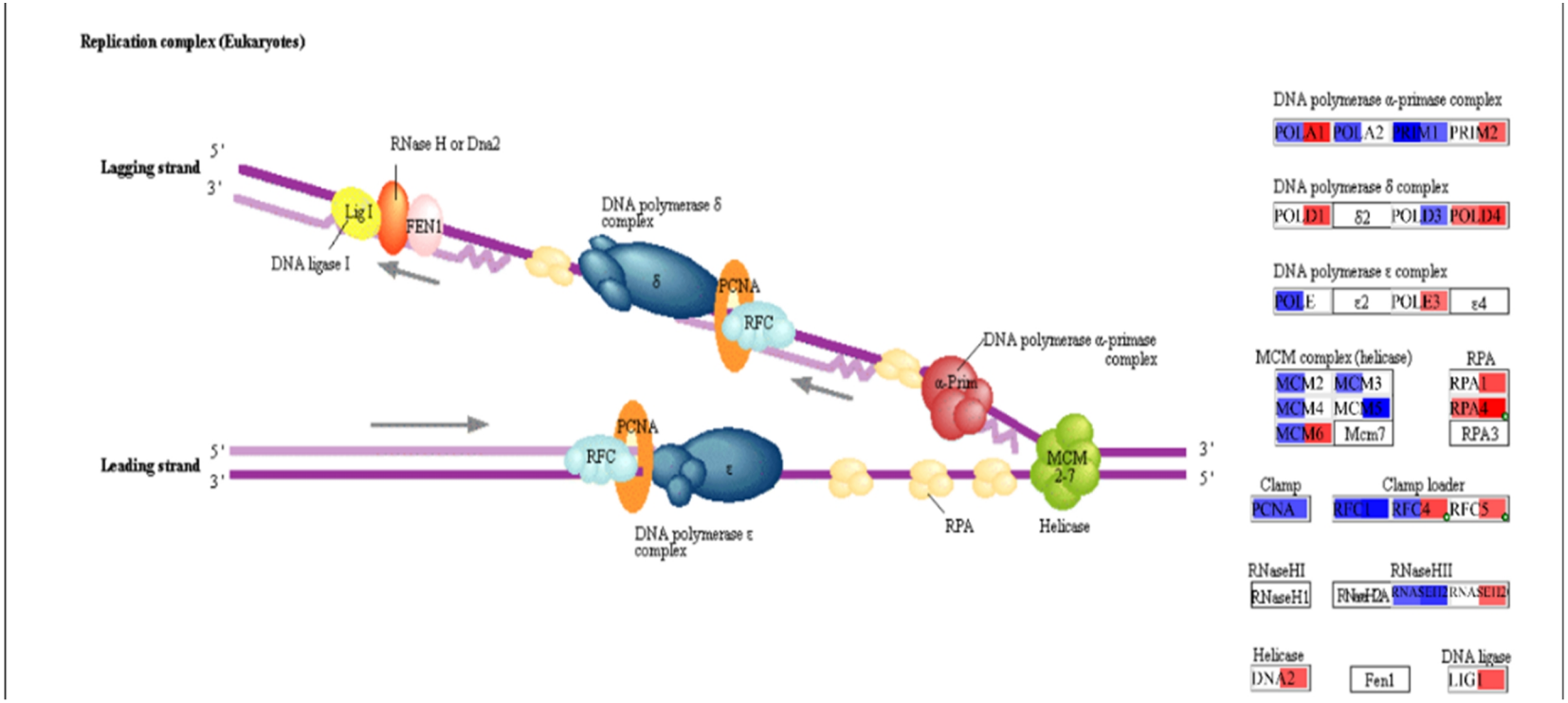


Image source: (Méchali, 2010)

From the gene expression data, it appears that in response to the stressful sea voyage environment [BL-P1], there should have been higher than usual DNA damage instances. Despite that the body when let alone (control group), promotes cell replication simultaneously having RPA4 and Polε checkpoints to ensure fidelity, repair DNA and maintain genomic integrity. Simultaneously Caspase and p53 mediated apoptosis pathways were triggered. Whereas, Yoga practice was able to enable a G2/M cell cycle arrest and also have RPA4 mediated checkpoint to address DNA damage, thereby probably reducing the number of errors. On the contrary, in control group, the CDK [CDK2, CDK6] that promotes replication and facilitates G1-S phase transition promoting cell proliferation were expressed (Bertoli, Skotheim, & de Bruin, 2013). It is noted that both ATM and ATR mediated DNA checkpoint pathways were downregulated in yoga group whereas they were upregulated in the control group. Upregulated ATM and CDC7 might also be a positive feedback mechanism to inhibit initiation of replication(Costanzo et al., 2003) in the control group. These data indicate that,

the cell proliferation and cell cycle was arrested in the Yoga group just through systematic downregulation of all genes associated with replication and simultaneously have RPA4 mediated DNA damage checkpoints. Whereas, in control group, it appears that there were multiple contradicting stimuli to promote replication, repression, DNA repair and apoptosis which might not be an efficient way to facilitate adaptation.


CELL CYCLE
Growth factor
Growth factor withdrawal
MAPK signaling pathway
GSK3B
p107
E2F4,5
E2F5
TGFB
TGFB1
Smad2,3
SMAD2
MYC
Miz1
SCF
SKP2
CDKN2A
Mdm2
Rb
TP53
CREBBP
PRKDC
ATM
DNA damage checkpoint
Apoptosis
p16
Ink4a
p15
Ink4b
p18
Ink4c
p19
CDKN2D
p27,57
CDKN1B
p21
Cip1
GADD45G
SFN
CHEK2
PCNA
Cdc25A
R-point
(START)
CCND3
CDK6
CCNE1
CDK2
CycA
CDK2
CycH
CDK7
CycA
CDK1
CCNB1
CDK1
Plk1
CDC25B
YWHAE
SKP1
Skp2
RBL2
Rb
ABL1
HDAC
Cdc6
CDC45
ORC2
MCM6
CDC7
DBF4
E2F4,5
DP-1,2
E2F2
TFDP2
Rb
Wee
PKMYT
Mps1
MAD1L
MAD2L
BubR1
BUB3
Bub1
SMC1A
SMC3
STAG1
Rad21
Cohesin
ESPL1
Separin
PTTG1
Securin
ANAPC
CDC20
Ubiquitin mediated proteolysis
APC/C
FZR1
CDC14A
Bub2
MEN
ORC (Origin Recognition Complex)
Orc1
Orc2
Orc3
Orc4
Orc5
Orc6
MCM (Mini-Chromosome Maintenance) complex
Mcm2
Mcm3
Mcm4
Mcm5
Mcm6
Mcm7
DNA
S-phase proteins, CycE
DNA
DNA biosynthesis
G1
S
G2
M


Replication complex (Eukaryotes)
Lagging strand
5'
3'
RNase H or Dna2
Lig I
FEN1
DNA ligase I
DNA polymerase δ complex
δ
PCNA
RFC
DNA polymerase α-primase complex
α-Prim
Leading strand
5'
3'
PCNA
RFC
ε
DNA polymerase ε complex
MCM 2-7
RPA
Helicase
3'
5'
DNA polymerase α-primase complex
POLA1 POLA2 PRIM1 PRIM2
DNA polymerase δ complex
POLD1 δ2 POLD3 POLD4
DNA polymerase ε complex
POLE ε2 POLE3 ε4
MCM complex (helicase)
MCM2 MCM3
MCM4 MCM5
MCM6 Mcm7
RPA
RPA1
RPA4
RPA3
Clamp
PCNA
Clamp loader
RFC1 RFC4 RFC5
RNaseHI
RNaseH1
RNaseHII
RNaseH2A RNASEH2B RNASEH2C
Helicase
DNA2
Fen1
DNA ligase
LIG1

4. ***Circadian Rhythm Regulation:***

Circadian rhythm regulation is the internal time keeping mechanism that exists in most of the tissues. Circadian rhythm is understood to intricately regulate metabolism, physiology and behavior and its dysregulation is associated with diseases (Green, Takahashi, & Bass, 2008). *CLOCK* and *BMAL1 [ARNTL]* gene form a heterodimeric factor complex to activate transcription of *Period (Per)* genes and *Cryptochrome (Cry)* genes. Activation of casein kinase Iε (*CSNK*) inhibits *CLOCK/BMAL1* transcription activity resulting in repression of *Cry* and *Per* genes.

Our results show that there was repression of *BMAL1* expression in the Yoga group in initial phase and an overexpression in the later phase of the expedition. Repression of BMAL1 in yoga group between initial phase might be an adaptation response to promote better insulin sensitivity (Moynihan Ramsey, Marcheva, Kohsaka, & Bass, 2007; Rudic et al., 2004) and its overexpression in Antarctic cold climate might be associated with increased lipogenesis (Shimba et al., 2005). Overexpressed *Per2* with repressed *c-Myc* in the control group might limit leptin dependant sympathetic regulation of bone formation in the cold Antarctic environment (Gimble, Zvonic, Floyd, Kassem, & Nuttall, 2006). More investigations are required to ascertain the regulation of circadian rhythm in cold Antarctic environment in both the groups. No contrasting regulation was noted.

5. ***Signal Transduction pathways***

Glycoseaminoglycans, heparan sulphate and hyaluronan participate in several biological processes including cell-matrix interactions and activation of chemokines, enzymes and

growth factors. Recent studies indicate several roles of glycosaminoglycans [GAG] and heparan sulphate [HS] in maintaining homeostasis and regulating inflammation other than the earlier known extracellular matrix component function (Taylor & Gallo, 2006; Whitelock & Melrose, 2011). A contrasting regulation was observed in NFκB signaling for the entire expedition duration: increased NFκB expression in the control group and an observation similar to previous reports of yoga repressing NFκB signaling (Acevedo, Pospos, & Lavretsky, 2016; Black et al., 2013) were noted.

In the initial phase of expedition, in both the groups, a mutual overexpression of *AGRN, GPC4* and *HSPG2* associated HS-GAG biosynthesis and degradation genes along with upregulated inflammatory response pathway genes: *CD86, THBS1 and LAMB1* were noted. Upregulated intergrin signaling along with CD-28 dependent PI3K-Akt signaling pathways and *VEGFR2* mediated increased vascular permeability were common between both the groups.

Interestingly, apart from the common pathways, yoga group had upregulated evolution conserved mTOR and Wnt signaling pathways. These pathways might be essential in regulating T cell homeostasis and determining cell fate (Chi, 2012; MacDonald, Tamai, & He, 2009; Peter, Waldmann, & Cobbold, 2010). A novel Apelin signaling pathway was found to be upregulated in the Yoga group. Apelin is a peptide hormone released by adipocytes in response to insulin levels (Boucher et al., 2005). Apelin in interaction with G-protein apelin coupled receptor is implicated in increasing glucose utilization and beta oxidation and lipolysis thereby promoting anti-diabetic and anti-obesity effects (Than et al., 2012; Yue et al., 2011). Apelin signaling pathway might contribute in maintenance of vascular smooth muscle cells and neurons (Chun et al., 2008; Foussal et al., 2010; Zeng, Yu, Zhang, & Wei, 2010) and promote vaso-dilatation moderated by nitric oxide (Tatemoto et al., 2001). We speculate that

apelin might enhance the adaptation response to cold by promoting lipolysis and enhancing heat production in yoga group. Earlier studies have reported an increased blood pressure in Antarctica (Cugini et al., 1997). In our study, in the yoga group, genes associated with positive regulation of blood vessel diameter were up-regulated – promoting reduction in blood pressure – moderated by the release of nitric oxide.

During the later phase of the expedition in the control group, apoptosis and necrosis signalling were upregulated. As an effort to alleviate smooth muscle contraction, the genes associated with metabolism of prostaglandin – *GNG2* and *PTGR2* were upregulated and genes that promote smooth muscle control contraction – *GTTN* and *OXT* were downregulated. Oxytocin (*OXT*) gene apart from regulating smooth muscle contraction is a marker for human social behaviour, trust, tolerance and adaptation (Lee, Macbeth, Pagani, & Scott Young, 2009). We speculate that downregulation of *OXT* gene in control group might be associated with attitude towards aggression in the later phases of expedition. Macrophage differentiation was also downregulated.

In the later phase of the expedition, the yoga group had downregulated *cAMP* mediated signalling and *MAPK* activity. The genes associated with Th2 cell differentiation were downregulated indicating better adaptation to the Antarctic environment. Coagulation of blood is delayed in Antarctica (Hicks, 1965). Megakaryocyte formation was upregulated in Yoga group, which might promote better blood coagulation.

6. <u>TCA Cycle:</u>

TCA cycle was significantly enriched in the BL-P1 comparisons between the Yoga and Control group. The lipogenic enzyme(Mackall & Daniel Lane, 1977) pyruvate carboxylase [*PC*] was upregulated in both Yoga and Control groups. The gene Isocitrate dehydrogenase (NADP(+))

2 [*IDH2*] and isocitrate dehydrogenase 3 (NAD(+)) beta [*IDH3B*] that catalyses oxidative decarboxylation of isocitrate to 2-oxoglurate were repressed in Yoga and control group respectively. And, the genes that catalyse the conversion of 2-oxoglurate to succinyl-CoA was downregulated in the Yoga group and upregulated in the Yoga group.

From the results it appears that the TCA cycle is repressed in the Yoga group. The excess Acetyl-CoA produced as a result of glycolysis would be converted to fatty acids. With increased levels of pyruvate carboxylase [PC] in both the groups, we speculate that in response to increased need for energy in stressful voyage, an adaptive mechanism to enhance pyruvate cycling capacity would have been adopted. PC catalyses the first step of gluconeogenesis to form oxaloacetate, which is converted into phosphoenolpyruvate and transported through the mitochondrial membranes by the tricarboxylic acid anion carrier system for reconverting phosphoenolpyruvate into glucose.Isocitrate dehydrogenase enzymes belongs to two different enzyme sub-classes: one that utilizes NAD(+) as electron acceptor and the other with NADP(+). Interestingly in the model organisms it is shown that despite following the same process of oxidative carboxylation, the Isocitrate dehydrogenase enzyme sub-class NADP(+) only is capable of exhibiting feedback inhibition by its reaction products alpha-ketoglutarate and NADPH (Dubois et al., 1998). This observation rises a speculation that through presently unknown pathways, Yoga practices are capable of performing feedback regulation of metabolic processes.

The increased levels of cholesterol and triglycerides might be due to increased fatty acid biosynthesis from the end products of Glycolysis. The results suggest that in the initial phase of the expedition, both the groups had increased fat biosynthesis, which was repressed in the later phases of the expedition. However, the number of genes regulated were more for Control

group. Interestingly, the krebs cycle was repressed in Yoga group and overexpressed in the control group. As an alternate, Yoga group had upregulated pyruvate recycling to better utilize the resources and thereby generate more heat.

A novel paradigm on the mechanism of action of Yoga:

The present study was designed as an exploratory study to provide us definite directions to understand how Yoga works. Antarctica, was of special interest for its inherent stressful conditions. Though, the stressful conditions at Antarctica is greater and also different from mainland, the effect of an intervention and its mechanism of action in that environment is considered beneficial in several areas of application including space programs and defense operations.

Until now, most studies have concluded that Yoga works through regulation of autonomic nervous system, establishing a para-sympathetic nervous system and thereby better stress resistance and deeper relaxation. Innumerable studies have demonstrated this observation in multiple scenarios. However, it appears that yoga not only works by regulating the autonomic nervous system but has bigger roles in regulation and adaptation.

The characteristics mentioned in the traditional literature matches with the recent neuro-imaging studies. Unlike individuals with chronic psychological stress, regular yoga practitioners had better self-awareness and interospection – which enables better synchrony between the mind and body and facilitates response based on the prevailing environmental stimuli without bias from the past negative experiences. Also, studies indicate that there are more nerve fibres recruited for a given task and the speed of transition of nerve impulses are better in Yoga practitioners(Sarang & Telles, 2006; Telles, Singh, & Puthige, 2013). This critical adaptation for preventing chronic stress induced non-communicable diseases needs

further studies for objective validation. So, the incongruence between the mind and body with less or no awareness of the present environmental condition, reliving the past or anticipating the future is bound to increase stress and Yoga promotes awareness of the present environment and changes the hardwiring in the brain appropriately (ref).

In this study, we observed a steep increase in cholesterol in Yoga practitioners than the Non-Yoga practitioners. Interestingly, genes that promote cardioprotection and vasodilatation mediated by nitric oxide were upregulated. In response to stress, multiple signals for promoting and inhibiting replication, apoptosis, DNA repair and cell proliferation were observed in the control group, whereas, yoga practitioners had a systematic downregulation of the cell replication machinery, a checkpoint to ensure fidelity of genetic material and DNA repair pathways were seen in the Yoga group. Cell survival signals were promoted in the Yoga group whereas it was only apoptosis in the non-yoga practitioners. Genes involving adaptive immunity were upregulated in the initial part of and was downregulated in the latter part of the expedition. Similar to the earlier observation, the NFκB pathway was repressed in Yoga and was overexpressed in the Control group. Also, consistently there were less number of regulated genes in the yoga group at every timepoint comparison indicating efficient regulation. So, yoga practices were able to promote cell survival, DNA repair, metabolism, alleviate oxidative stress, inflammation and regulate cellular response to stress to facilitate shifting towards homeostasis.

Based on the present findings, earlier studies and the traditional literature, we observe a pattern of regulation unique to Yoga practices. To explain these multiple observations, we have coined a terminology – "*Intelligent Consciousness*". We define, *Intelligent Consciousness* as a paradigm that can perceive, understand and execute the right process at the right time,

appropriate for that environment, thereby making the process efficient resulting in optimal regulation.