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DOMINO SYSTEMS BIOLOGY APPROACH: ENERGY METABOLISM AND DNA DAMAGE IN *SACCHAROMYCES CEREVISIAE*

Malkhey Verma*

Department of Biochemistry and Microbial Sciences, Central University of Punjab Bathinda, Punjab, India.

ABSTRACT: A 'domino' strategy has been used to develop model starting with one prevailing feature of cell function as ATP and the major process producing it, and later increase additional processes and components bit by bit only as required to elucidate incited experimental observations. The approach is here applied to the energy metabolism of yeast growing on glucose, supplemented with methyl methanesulphonate (MMS), a DNA damaging agent. The mysteries resolved include (1) the lack of decrease in the adenosine triphosphate (ATP) upon addition of methyl methanesulphonate, (2) the lack of proportional increase in adenosine diphosphate (ADP) when ATP energy is used upon hydrolyzed, and (3) the fast disappearance of the adenine moiety of adenine nucleotides. Not either the incorporation of nucleotides into newly synthesized biomass nor steady state *de novo* synthesis of adenosine monophosphate (AMP) explains. Cells recycle their adenine moiety at a faster rate when energy state in endangered, is one of the essential domino features amongst the seven required for the understanding of the experimental observations. The current domino systems biology approach shows how tactical experimental planning and data in aggregation with theoretical concepts and mathematical simulations could recognize and decide about main puzzles. This approach also prevails the unforeseen role of the adenine component of ATP.

KEYWORDS: Adenine nucleotides, Steady state, Glycolysis, kinetic modeling, DNA damage, Methyl methanesulphonate, paradoxes.

Corresponding Author: Dr. Malkhey Verma* Ph.D.

Department of Biochemistry and Microbial Sciences, Central University of Punjab Bathinda, Punjab, India. Email: Malkhey.verma@cup.edu.in

1.INTRODUCTION

All eukaryotic cells are constantly exposed to exogenous or endogenous agents that damage DNA. DNA is highly reactive and easily altered either by normal cell processes or by exogenous factors. One estimate is that a mammalian genome undergoes about 100,000 modifications per day, each bearing a finite probability of residual damage [1]. Accumulation of unrepaired DNA damage can lead to increased levels of intracellular reactive oxygen species (ROS) which, among other things, further increases genetic instability. To cope with damaged DNA cells utilize a wide range of repair mechanisms necessary to halt the deteriorating effect of damaged DNA. Excessive DNA damage leads to the accumulation of mutations which are the basis for carcinogenesis in mammalian cells.

Growth arrest represents an adaptive and integral part of the cellular DNA damage response. It ensures the preservation of energy and reducing equivalents necessary for macromolecular stabilization and repair, and, what is also very important, gives enough time for the repair process. Proliferating cells that actively undergo DNA replication and mitosis are more prone to suffer damage to macromolecules than cells in a resting state [2]. Therefore, the activation of cell cycle checkpoints is the key mechanism in the prevention of further cellular damage. Connected with cell cycle arrest is the modulation of major pathways of energy metabolism, which may be closely linked to the oxidative burst in cells exposed to DNA damage. Induction of many key enzymes involved in glycolysis, pentose phosphate pathway, or the Krebs (citrate) cycle may be necessary for generating reducing equivalents (NADH, NADPH) that are needed for cellular antioxidant systems [2]. The elevated transcription of glycerol-3-phosphate dehydrogenase (G3PDH), 6 phosphogluconate dehydrogenase (6PGDH), enolase, citrate synthase, and isocitrate dehydrogenase (IDH) were observed in response to many stress conditions or DNA damage [3, 4, 5]. Moreover, growth arrest results in redirection of NADPH/NADH and ATP utilization from proliferative processes to macromolecular stabilization and repair [2]. The potential reason for inducing these metabolic pathways lies in the energetic requirements of protein degradation, protein chaperoning, and DNA repair. Despite our intense knowledge on the cellular response to various kinds of DNA damage, little is known on how cellular energy homeostasis is linked to DNA damage response and the ability of cells to cope with DNA lesions. It was known that the severity of oxidative damage to mitochondrial DNA is dependent on the activity of mitochondrial NADH kinases whose activity depends on the ATP concentration [6]. We also found that the sensitivity of yeast cells to the DNA alkylating agent methylmethanesulfonate (MMS) is altered in yeast strains lacking FBP1, the key enzyme of gluconeogenesis [7]. Furthermore, production of reactive oxygen species (ROS) in DNA damage conditions directly correlates with the alterations of mitochondrial function [8]. DNA damage repair is a process that is driven by ATP hydrolysis. As a consequence, the infliction of DNA damage to yeast cells through a genotoxic compound such as MMS would be expected to lead to a decrease in cellular ATP levels. Such a decrease in ATP levels could then compromise DNA damage

repair. Another consequence of DNA damage repair activation is the utilization of NAD by the chromosomal enzyme, Poly(ADP-ribose) polymerase (PARP) which transfers 50 –200 branched chains of ADP-ribose to a variety of nuclear proteins. The enzyme is chromatin-bound and usually exists in an inactive form. It is activated by DNA strand breaks to cleave NAD at the glycosylic bond between the nicotinamide and adenosine diphosphoribose moieties. Because NAD is required for enzymatic reactions involved in the generation of ATP, consumption of cellular NAD pools results in loss of ability to synthesize ATP, with consequent depletion of cellular ATP pools and loss of all energy-dependent functions. Taken together, ATP is essential for the repair of damaged DNA suggesting that energy homeostasis - monitored in the form of ATP concentration, the ATP/ADP ratio, or the adenylate energy charge - may play a vital role in the ability of (yeast) cells to respond to DNA damage as well as to other stress conditions. Modeling of energy metabolism in the DNA damage condition and related stress situations will help us to understand the regulatory mechanisms involved in energy balance homeostasis in DNA damaged cells and to explore this knowledge for future anti-cancer therapy. Most *in silico* systems biology modeling efforts are still restricted to specific parts of the metabolic map of the cell (e.g. glycolysis) or regulatory structure (e.g. glucose sensing signalling pathway). *In vivo*, however, these cellular sub-systems are highly integrated to form the complete living organism such as a yeast cell. The ultimate aim of systems biology must be to produce fully integrated kinetic models at the level of the whole organism. We developed a new Systems Biology approach, called domino Systems Biology, which builds up the entire story by beginning simple and adding modules and intermediates where necessary. Integrating the data from the DNA damage analysis into the domino system biology approach our aim was to investigate how other ATP producing/consuming processes might help maintain DNA damage repair by affecting the homeostasis of the ATP consumed by the repair process. The results obtained revealed significant alterations of the glycolytic flux and in the energy homeostasis of DNA damaged cells and provided a first experimental demonstration that the influence of genotoxic agents on cellular metabolism and survival depends on nutrient availability and energy metabolism. The domino systems-biology models developed ultimately could predict a sustained increase of intracellular ATP concentration, called “DNA repair paradox”, consequent to a reduction of growth, caused by the run-out of the carbon substrate. Moreover, the complex model that included all regulatory modules could better explain how those modules are integrated together to lead to cell function in stress and damage challenging conditions.

2. MATERIALS AND METHODS

Model Development

DNA Damage Induction

MMS (methyl methanesulfonate) was chosen as the first DNA damaging agent to analyze in terms of its influence on energy metabolism. MMS treatment of S. cerevisiae clearly leads to a growth

arrest. This is connected to the Rad9/Mec1 recognition of damaged DNA involving stalled replication fork, which leads to activation of Rad53 and hence cell cycle arrest [9]. When Rad9 is deleted the MMS mediated growth arrest is suppressed. Also, mitochondrial DNA is targeted by MMS, which in mammalian cells is known to trigger the mitochondrial apoptotic pathway. Methyl methanesulfonate (MMS) is a methylating agent that methylates DNA at 7-deoxyguanine and 3-deoxyadenine. The resulting 3-methyladenine (3MeA) and 7-methylguanine cause base mispairing and replication blocks which activate DNA damage repair pathways and cell cycle arrest [10]. The major repair pathways involved in the repair of DNA alkylation damage are predominantly base excision repair (BER) and repair by DNA alkyltransferases [11], but all three radiation repair pathways are involved in this process as well [12]. In yeast Mag1p (3MeA DNA glycosylase) removes the damaged base, than Apn1p (apurinic/apyrimidinic endonuclease), cleaves the DNA strand at the abasic site for following repair [1]. Overexpression of MAG1 causes a mutator phenotype [13] as does mutation of APN1 [14], suggesting that this AP site produced by Apn1p is the first product of lesion processing that can be converted to a potentially lethal double-strand break (DSB) and checkpoint activation [13]. In addition, MMS was shown to cause oxidative cell injury that follows the depletion of intracellular glutathione (GSH) [15]. A decrease in the intracellular pool of reduced GSH results in an increase of ROS levels. The latter, of course, depends on the balance of ROS production versus ROS scavenging. However, MMS is not expected to directly cause intracellular ROS formation [16]. More likely, ROS may function as a signal which mediates cellular response to unprepared DNA damage. Therefore, cellular response to MMS treatment includes a complex network of proteins involved in DNA repair, cell cycle control, oxidative stress response, and apoptosis. The magnitude of response will, of course, depend on the dose and time of treatment. Intracellular ATP level is an important determinant of signalling cascades and cell fate upon DNA damage induction. At sites of DNA damage, chromatin structure is altered to expose damaged DNA to repair factors, and once the repair has taken place, chromatin is restored to its original state. Chromatin remodeling factors are highly ATP-dependent and depletion of intracellular ATP level can inhibit DNA repair and induce apoptosis. However, how ATP level triggers survival and death pathways is still not entirely clear. It was also shown that several antitumor and antibacterial drugs induce a rapid elevation in the total pool of the NTPs in prokaryotes and eukaryotes [17, 18, 19]. This increase appeared to be associated with an apoptotic-like process providing a signal for endonucleolytic fragmentation of DNA and subsequent cell death [17, 19]. To model cellular ATP level upon treatment with DNA methylating agent MMS and estimate the relationship between cellular energetic state and DNA damage we developed DNA repair model based on domino systems biology approach, postulating that ATP level is determined by a complex interaction between ATP consuming and ATP producing pathways.

Domino Systems Biology Approach

The 'Domino' approach is the building of a comprehensive model by starting with a simple model for the core target of the problem to explain the biological paradoxes [20]. We have started our system biology approach from DNA repair model and added various modules bit by bit. This approach helps to understand the effect of the newly added module on the model and also give insights for the next module to be added. We have begun from DNA repair module and increased modularity till we reached to the explanation for the descovered paradoxes of ATP and AXP. The domino approach was developed in the following sequence: DNA repair module per se as the first model (Figure.1A). Next, glycolysis and maintenance modules were integrated into the DNA repair model as ATP producing and ATP consuming components, respectively (Figure.1B). Further, the growth component was added to the glycolysis, maintenance, and DNA repair modules as the ATP consuming module (Figure.1C). Finally, nucleotide synthesis was assigned to the glycolysis, maintenance, growth, and DNA repair integrated model as an additional ATP consuming module (Figure.1D).

The Modules

A domino series of dynamic models for yeast was developed by integrating different ATP producing and consuming modules step-by-step [20]. These modules are always linked by their interaction with the adenine nucleotide pool consisting of ATP, ADP and AMP and we, therefore, treat these concentrations as global variables of total dimension 2, assuming adenylate kinase equilibrium. Some of these modules may represent detailed published models of metabolic pathways whereas others may be simpler minimal models describing the gross behaviour of that sub-system. The overall model should capture the most important interactions between the modules. ATP is perhaps the most obvious 'hub' of interaction since it is the main energy carrier of the cell the production of which from ADP is driven by catabolic processes and which drives anabolic processes such as mRNA and protein synthesis and maintenance. Thus ATP is constantly being 'turned over' to ADP and back.

Glycolysis Module

For this module, we have used the Teusink model of glycolysis [21] with an explicit representation of ATP, ADP and AMP. We have replaced ATPase flux by maintenance and growth at a steady state with the glucose concentration of 50 mM.

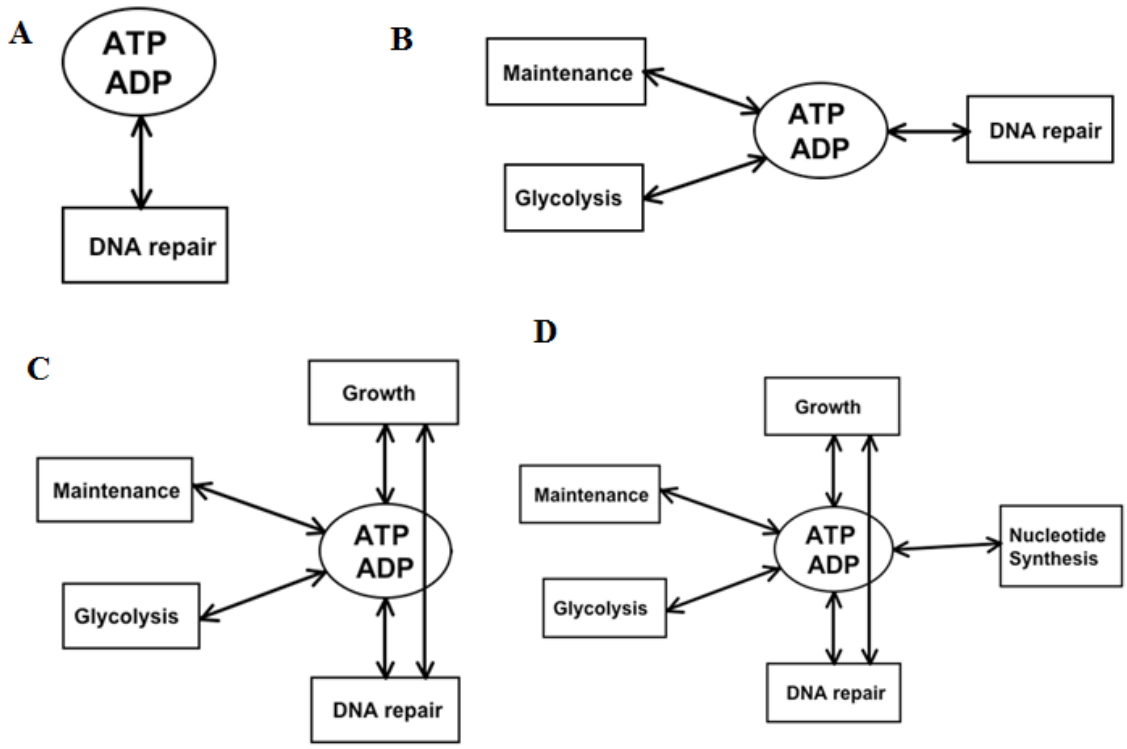

Figure 1. Domino modular approach of model development. (A). Represent ATP integration and hydrolysis for the DNA repair. (B). Representation of ATP production by glycolysis and consumption in DNA repair and maintenance. C. Schematic of the addition of ATP consuming growth module to model B. (D) Schematic, the addition of new feature Nucleotide Synthesis in the model (C) [20].

Maintenance Module

This process consumes ATP energy to keep cellular machinery in the working state. Maintenance needs 20% of ATP consumed for growth [21, 22]. The consumption of ATP is its turnover to ADP and Pi.

Growth Module

The version-2 of growth is also a minimal model consisting of a single flux consuming ATP and producing ADP. The rate equation assumes irreversible Michaelis-Menten kinetics for the substrate (ATP) with competitive inhibition by the product (ADP) and regulated allosterically by glucose-6-phosphate as shown in Equation. (1).

$$v^{\text{growth}} = \mathrm{V}_{\text{max}}^{\text{growth}} \left(\frac{\frac{[\text{ATP}]}{\mathrm{K}_{\text{m,ATP}}^{\text{growth}}}}{1 + \frac{[\text{ATP}]}{\mathrm{K}_{\text{m,ATP}}^{\text{growth}}} + \frac{[\text{ADP}]}{\mathrm{K}_{\text{i,ADP}}^{\text{growth}}}} \right) \left(\frac{G6P^3}{K_{sG6PGrowth}{}^3 + G6P^3} \right) \quad (1)$$

One key aspect of this flux in both versions of growth is that the ratio of ADP production to ATP consumption is slightly less than unity ($=1-\rho_{\text{incorp}}$) with the ratio of ATP incorporation into biomass

to ATP turnover $\rho_{incorp} = 0.01$. This can be seen in the nucleotide balance equations. The ATP flux from glycolysis module was split to maintenance and growth modules. The ATP flux for maintenance is kept constant and the value of V_{max}^{Growth} is calculated by balancing the out coming ATP flux from glycolysis and consumption of ATP flux by maintenance and growth modules in the glucose-rich condition. The G6P node in the glycolysis is highly connected to various biosynthetic pathways (pentose phosphate pathway/salvage pathway, amino acid, glycogen, and trehalose biosynthesis) those directly contribute for the cell growth so G6P regulation is incorporated. The exponent of G6P is calculated to make growth sensitive for the external glucose level. The value for ρ_{incorp} is calculated using the data in Table 1. Here we assume that all the ATP is incorporated into either RNA or DNA and that, one ATP is incorporated for each nucleotide base. We use an average value for the molecular weight of the nucleotides making up RNA and DNA of 330 daltons per nucleotide. We take a typical yield for the grams of biomass produced per gram of glucose for a chemostat with a dilution rate of 0.25 h^{-1} [23]. We take values of 0.073 and 0.003 for the RNA and DNA dry-mass fractions of baker's yeast respectively [24].

Table 1: Data for calculation of ATP incorporation fraction in growth

Property	**Value**	**Unit**	**Reference**
Glucose mol wt	180	g/mol	Standard
Average RNA/DNA base mol wt	330	g/mol	Calculation
moles ATP per mole glucose (pure fermentation)	2	Mol/mol	[27]
Typical yield (g biomass per gram glucose)	0.48	g/g	[28]
RNA composition of yeast (g RNA per g biomass)	0.073	g/g	[28]
DNA composition of yeast (g DNA) per g biomass)	0.003	g/g	[28]
Moles ATP incorporated per moles ATP turnover	**=(0.48*(0.073+0.003)/330)/(2/180) = 0.00995**	**Mol/mol**	Model calculation for anaerobic condition

These values are similar to those reported more recently, i.e. 0.063 and 0.004 respectively [25] which have been used in genome-scale models of yeast metabolism [26]. I neglect respiration and associated ATP synthesis and that net production of ATP is 2 moles per mole of glucose. If respiration is significant then the effective ATP production per mole of glucose will be higher and the calculated incorporation fraction will be lower.

Nucleotide Synthesis Module

It is clear that cell growth will gradually deplete the nucleotide pool over time. Therefore a module that replenishes the pool – i.e. nucleotide synthesis module was introduced. This is another minimal

model in which we use a single equation to model the *de novo* synthesis of AMP. We ignore substrates and (rather inaccurately) the energy (ATP) requirement of this synthesis. Rather, we merely assume that the reaction is fixed rate, and inhibited by end product ADP and ATP as activity of the first enzyme has feedback inhibition, inhibited by end product ADP and ATP in the *de novo* pathway [27].

$$v^{\text{nucleotide\_synthesis}} = \left(\frac{V_{\max}^{nucleotidesyn}}{1 + \frac{[ADP]}{K_{iADP}^{nucleotidesyn}} + \frac{[ATP]}{K_{iATP}^{nucleotidesyn}} + \frac{[ADP][ATP]}{K_{iADP}^{nucleotidesyn} K_{iATP}^{nucleotidesyn}}} \right) \quad (2)$$

The Vmax value for the nucleotide synthesis is calculated by at glucose-rich steady state by balancing the rate of AMP synthesis and the rate of incorporation of ATP into biomass since these are the only reactions that are net consumers/producers of the adenine nucleotide pool. The K_{iADP} and K_{iATP} are taken equal to ADP and ATP concentrations in glucose-rich conditions.

Intracellular transport of MMS

The transport of methyl methanesulphonate (MMS) from the bulk of the medium to the intracellular environment is by passive diffusion which we assume to be proportional to the concentration difference across the cell membrane (the constant of proportionality is the membrane permeability coefficient for the MMS).

$$v_{\text{passive}}^{\text{influx}} = k_{\text{perm}}^{\text{influx}} ([\text{Drug}_o] - [\text{Drug}_i]) \quad (3)$$

DNA damage and repair module

MMS follows the SN2 mechanism for the methylation of double-stranded DNA (dsDNA) *in vitro* [29]. Consequently, our model assumes a similar mechanism for *in vivo* DNA damage afflicted on *Saccharomyces cerevisiae*. The methylation of dsDNA by MMS and rate expression are shown below;

$$DNA + MMS = \text{DNA}_{\text{damaged}} \quad (4)$$

$$v_{\text{DNA\_damage}} = k_2[DNA][MMS] \quad (5)$$

The model assumes that the methylated DNA could not be replicated, which reduces the cell growth by a factor of fraction of the accumulation of methylated DNA.

$$\frac{[DNA_{damaged}]}{[DNA] + [DNA_{damaged}]} \quad (6)$$

So the final form of growth expression is

$$v^{\text{growth}} = \mathrm{V}_{\max}^{\text{growth}} \left(\frac{\frac{[\mathrm{ATP}]}{\mathrm{K}_{\mathrm{m,ATP}}^{\text{growth}}}}{1 + \frac{[\mathrm{ATP}]}{\mathrm{K}_{\mathrm{m,ATP}}^{\text{growth}}} + \frac{[\mathrm{ADP}]}{\mathrm{K}_{\mathrm{i,ADP}}^{\text{growth}}}} \right) \left(\frac{G6P^3}{K_{sG6PGrowth}{}^3 + G6P^3} \right) \left(\frac{DNA}{DNA + DNA_{damaged}} \right) \quad (7)$$

here the cellular concentration of DNA is taken as the concentration of nucleotide in the yeast genome. By considering cell volume 70 cubic μm [28, 30-33] the nucleotide concentration was calculated 0.6 mM as shown in Table 2. The alkylated nucleotides in DNA are repaired by the base excision mechanism [34]. The position of the alkylated nucleotide can be recognized by protein DNA glycosylate and then removed. The DNA polymerase I fills the gap by new nucleotide and join the DNA strands by DNA ligase hydrolyzing one ATP to ADP. Model makes an assumption that the all the nucleotide monophosphate (NMP and dNMP), nucleotide diphosphate (NDP and dNDP) and nucleotide triphosphate (NTP and dNTP) are equivalent to AMP, ADP and ATP respectively. The important aspect of ATP flux for DNA repair is that the ratio of ADP production to ATP consumption is less than unity. There is the incorporation of one ATP and turnover of another ATP to ADP for each nucleotide repair. The stoichiometry of ATP and ADP is shown in the repair reaction (8).

$$DNA_{damaged} + 2ATP = \mathrm{DNA} + \mathrm{ADP} \quad (8)$$

The DNA repair is modeled using irreversible Michaelis-Menten kinetics as follows;

$$v^{DNArepair} = \mathrm{V}_{\max}^{\mathrm{DNArepair}} \left(\frac{[\mathrm{DNA}_{\mathrm{damage}}][ATP]}{K_{mDNA_{damaged}}^{DNArepair} \times K_{mATP}^{DNArepair}} \right) / (1 + \frac{[DNA_{damaged}]}{K_{mDNA_{damaged}}^{DNArepair}} + \frac{[ATP]}{K_{mATP}^{DNArepair}}) \quad (9)$$

Table 2: Data for calculation of DNA (nucleotides) concentration in the *Saccharomyces cerevisiae*.

Property	Value	Reference
Genome size (number of base pairs in the genome)	12 million base pairs	Database of Genome Sizes (Center for Biological Sequences Analysis)
Number of nucleotides/cell	2.4×10^7	Calculation
Volume of cell	70 μm	[28, 30-33]
Volume of cell	7.0×10^{-14} litre	[28, 30-33]
Avogadro number (N)	6.023×10^{23}	Standard
Number of moles of DNA (nucleotides)/cell	~ 4.0×10^{-17} moles	Calculation
DNA (nucleotides) concentration	~ 0.6 mM	Calculation

Parameters

The parameters used for the model are given in Table 3. The parameters have been manually fitted so that the steady state fluxes and the ATP concentration for the first four modules are similar (note however the large differences in ADP and AMP); and also so that the fifth module (with growth and drug efflux) and sixth module (DNA damage and repair) shows quantitative agreement with the results obtained for benzoic acid efflux and DNA repair are discussed below.

Table 3: Parameter values used in the model

Parameter	Value	Source
V_{max}^{growth}	165 mM min^{-1}	Based on parameter sensitivity
$K_{m,ATP}^{growth}$	1 mM	Based on parameter sensitivity
$K_{i,ADP}^{growth}$	1 mM	Based on parameter sensitivity
K_{sG6P}^{growth}	0.5 mM	Based on parameter sensitivity
ρ_{incorp}	0.01	Model calculation
$V_{max}^{nucleotide\_synthesis}$	4.03 mM min^{-1}	Based on parameter sensitivity
$K_{i,ADP}^{nucleotidesyn}$	1 mM	Based on parameter sensitivity
$K_{i,ATP}^{nucleotidesyn}$	2 mM	Based on parameter sensitivity
$K_{sG6PGrowth}$	0.5 mM	Based on parameter sensitivity
k_{perm}^{influx}	0.08 min^{-1}	Based on parameter sensitivity
$K_{maintenance}$	19.81	Model calculation
$k_{2DNAdamage}$	0.003 $mM^{-1}min^{-1}$	Based on parameter sensitivity
$K_{mDNAdamaged}$	1 mM	Based on parameter sensitivity
$K_{mATPDNArepair}$	1 mM	Based on parameter sensitivity
$V_{max}^{DNArepair}$	0.013 mM min^{-1}	Based on parameter sensitivity
[DNA]	0.6 mM	Model calculation

Experimental data

All **t**he experimental data of energy metabolism for control and after MMS treatment in the yeast strain; FF 18984 (*MATa leu2-3,112 ura3-52, lys2-1, his7-1*). Yeast cells were grown in rich (YPD) medium and various other mediums were extracted from Kitanovic et al., [35] by using XY-extract digitizer software.

Energy Charge

Energy charge is mathematically defined as the ratio of the sum of hydrolysable phosphate bonds in adenine nucleotides to the sum of total concentration of nucleotides (AXP), calculated using this formula ([ATP]+0.5[ADP])/([ATP]+[ADP]+[AMP]) [36].

3. RESULTS AND DISCUSSION

Kitanovic et al., have measured a number of indicators of the cellular energy state, notably ATP, ADP and AMP, to see if these were affected by DNA damage and/or repair [35]. Looking at DNA damage and repair alone, as in Figure.1A, or in a system with glycolysis and maintenance (Figure 1 B). I would expect that the addition of MMS should lead to a decrease in ATP and an increase in ADP or AMP such that the adenylate energy charge should drop. In terms of our central question, data showed that MMS treatment clearly and significantly affected cellular ATP levels, as expected [35]. However, for cells in rich medium (YPD) we detected an increase in ATP level, rather than the expected decrease, in the first hours after adding MMS to the cell culture. Surprisingly this was accompanied by a concomitant increase in ADP or AMP, and indeed sumAXP (ATP+ADP+AMP) also increased upon addition of MMS. Only at later time points both ATP and ADP contents were decreased significantly. I identified the increase in ATP level upon infliction of DNA damage as a paradox. Clearly, we would expect damage to induce repair, which is an ATP hydrolyzing process. Also otherwise additional damage was likely to lead to a drop in the cellular energy state and not an increase. I decided to call it the 'ATP-repair' paradox and make it a test case for the development and success of domino systems biology. The paradoxical changes of ATP content in YPD medium were also found in control cultures at the transition from exponential growth to stationary phase. I had expected that the ATP content would be highest and time-independent in the exponential growth as S. cerevisiae cells rapidly consume large amounts of glucose, while in stationary phase glucose is exhausted and cells rely on their storage carbohydrates or non-fermentative carbon sources, with reduced ATP levels as a result. However, experimental results [35] revealed that cellular ATP content was not at its maximum in the fast-growing exponential phase: it increased upon transition to the stationary phase and the concomitant reduction and then of growth rate. The ADP content, and by this the AXP content as well, also showed a progressive increase with the reduction of growth rate and upon entering of the stationary phase. As a result, the AXP content (mostly attributed to ATP and ADP levels) was the highest in non-growing stationary cells. Realizing that ATP changes almost lead to ADP changes, in reality, I took the ATP/ADP ratio or rather the adenylate energy charge (ATP+0.5ADP)/(ATP+ADP+AMP) (these properties are interrelated in our models because we assume that the adenylate kinase reaction is always at equilibrium) as the first central intermediate. Our first module was DNA damage plus the consequent ATP hydrolyzing repair. The results of first model simulations confirmed that with this DNA damage and repair module alone, in interaction with ATP and ADP (Fig.1 A), MMS would induce a sustained decrease in ATP levels, obviously

because ATP would be hydrolyzed continuously in the absence of a source of new ATP (Figure 2). We then added the modules for glycolytic ATP production and for ATP hydrolyzing maintenance metabolism from the Teusink et al. [21] detailed kinetic model of glycolysis. This new domino model (schematized in Figure 1 B) still predicted that MMS addition should lead to a decrease in ATP concentration. We next added a cell growth module, where cell growth depended on the concentration of ATP and glucose-6-phosphate. Not even with this new domino model an increase in ATP upon MMS addition was predicted (Figure. 3). In the next development, we concluded that we should perhaps include the effect of DNA damage on specific growth rate through the inhibition of the progression of the DNA replication forks. When we added this extra and direct interaction between the DNA damage/repair module and the growth module, the domino model began to predict a minor increase in ATP level, not as strong as observed experimentally but still qualitatively the same (Figure 3).

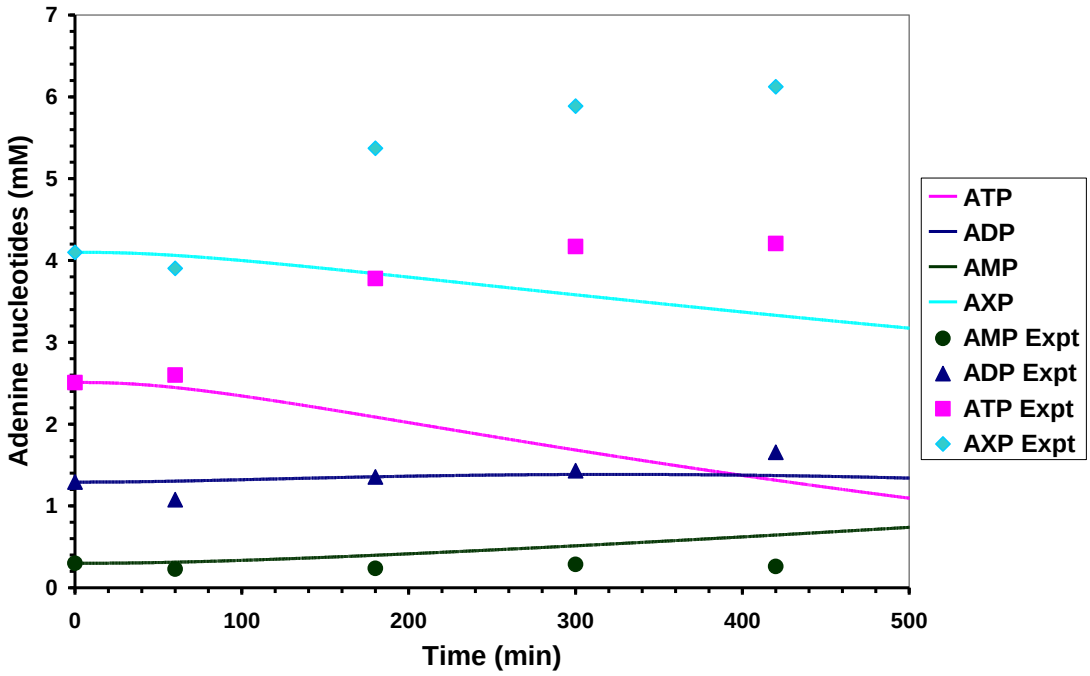

Figure 2. Evaluation of experimental data of adenine nucleotides with simulations of the model with adenine nucleotides pool and DNA repair module (Figure 1A).

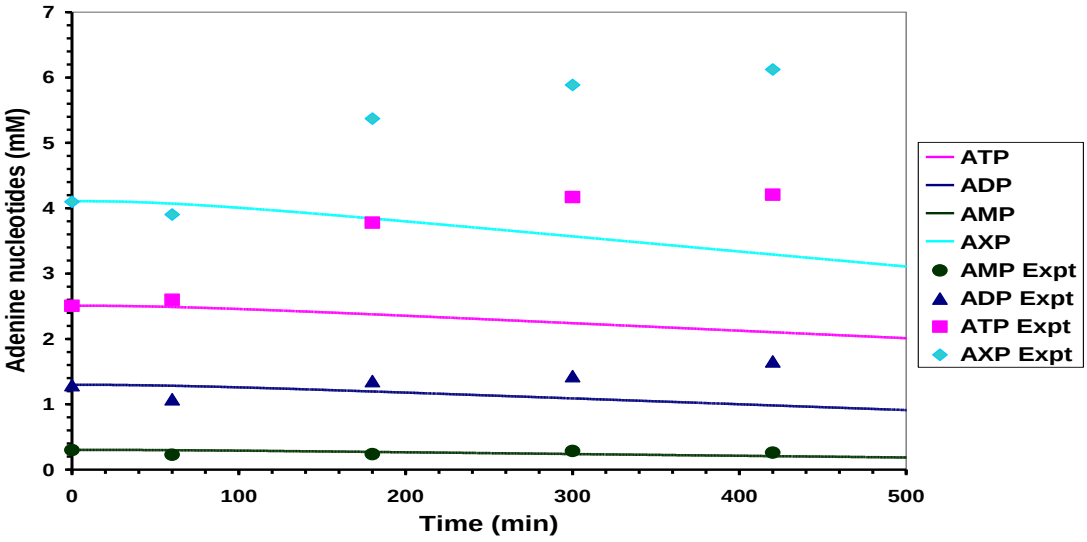

Figure 3. Comparison of experimental data with model simulations of the model having glycolysis, growth, maintenance, and DNA repair modules (Figure 1C).

Based on developments in dealing with the dynamics of adenine nucleotide concentrations after addition of excess glucose to a glucose-limited chemostat [20], we paid attention to the time dependence of the sum of the adenine nucleotide concentrations [20]. we noted in the experimental results that those sum concentrations also increased quite significantly upon addition of MMS. This made us implement the growth module model that took into account the incorporation of adenine nucleotides into new biomass. Because at steady state this leads to a net consumption of adenine nucleotides, a reaction producing adenine nucleotides had to be added as an extra module (Figure 1 D). With the domino systems biology approach model produced, we could simulate an increase in ATP as well as an increase in total adenine nucleotide concentrations soon after the addition of MMS, in line with the experimental observations (Figure 4 A & B).

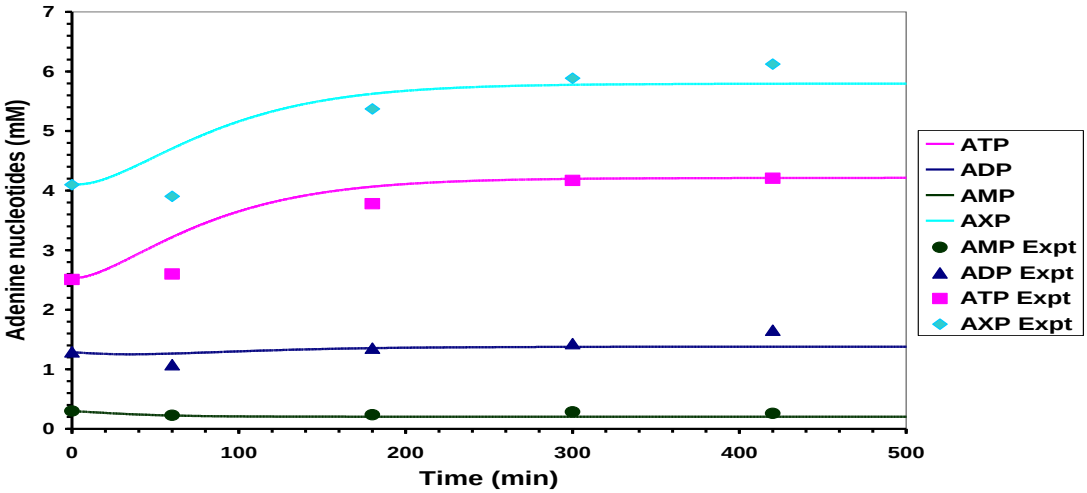

Figure 4 A. Comparison of experimental data and simulations results of the model having glycolysis, maintenance, growth, nucleotide synthesis and DNA repair modules for adenine nucleotides.

Although not just a perfect simulations of the measured data – it must be considered that the equations used are only a first approach – the model already successfully describes the paradox of ATP (AXP) increase in stationary phase upon glucose exhaustion in control samples. The developed models could also predict a sustained increase of intracellular ATP (AXP) concentration concomitant with the reduction of growth that occurred at 5 h because of glucose depletion [35] (data not shown). This is followed by an increase of ADP and AMP levels (data not shown). The results are in agreement with the obtained results implicating that the ATP-consuming pathways, like biomolecule synthesis, are strongly stimulated in exponentially growing cells and further down-regulated with the reduction of growth rate and nutrient (preferentially glucose) exhaustion.

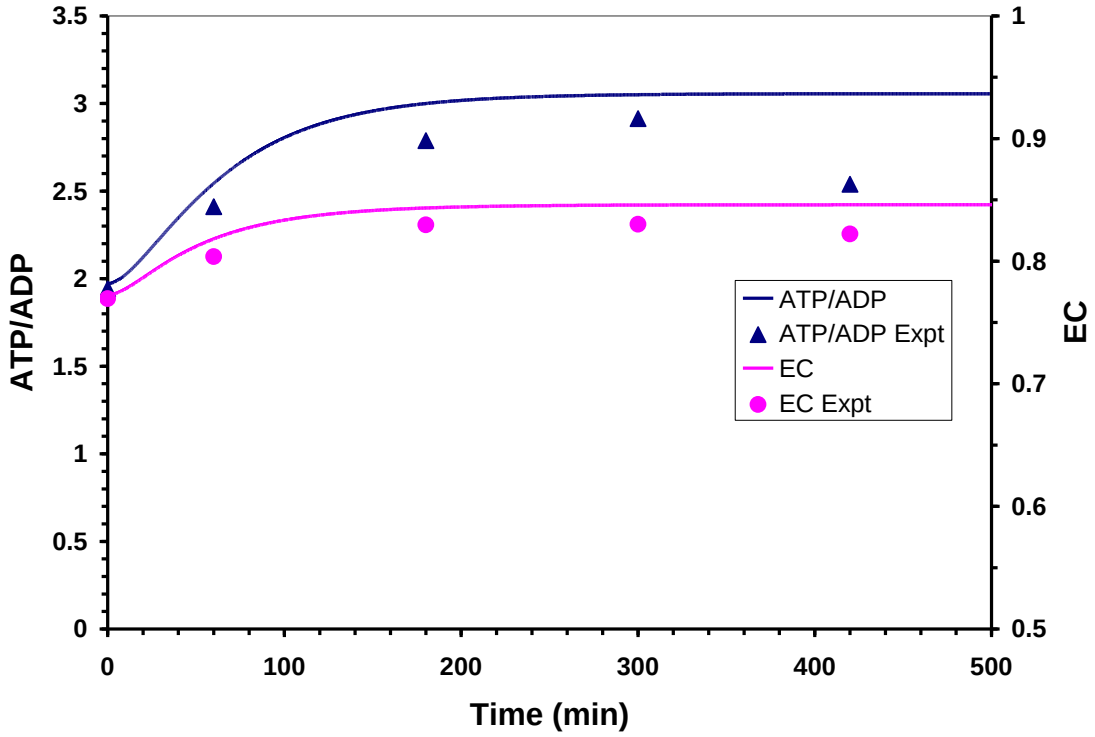

Figure 4 B. Comparison of experimental observations and simulation results of the model with glycolysis, maintenance, growth, nucleotide synthesis and DNA repair modules for ATP/ADP ratio and energy charge (EC).

4. CONCLUSION

In this paper, we have put this all in a complete and 'domino' perspective and have shown that Saccharomyces cerevisiae growing in the glucose-rich conditions and the growth of yeast and the subsequent addition of MMS can be understood in terms of a single model. The domino approach of systems biology examines in the modular form of the model development: the iteration of the model to experiment is a reversible process and getting into a spiral into the third aspect of understanding, where each cycle of the model development is compared with latest experimental observations or creates the requirement of the further experiments. Another turn of the spiral should

locate the involvement of the internal carbon signal (pyruvate and fructose bisphosphate were initial proposals), and the redox element. This approach could be used to understand cancer cells' constitutively activation of the uptake and metabolism of nutrients that both promote cell survival and fuel cell growth. Cancer cells incline to "ferment" glucose into lactate even in the presence of sufficient oxygen to support mitochondrial oxidative phosphorylation. Recent studies showed that reliance of cancer cells on glycolytic metabolism and impaired mitochondrial function, the so-called Warburg effect, is results of high requirements of fast proliferating cells for production of acetyl-CoA and NADPH important precursors and ATP for macromolecular biosynthesis. The metabolic dependencies of malignant tumors can be exploited for cancer treatment by combined DNA damage and ATP depletion therapy.

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CONFLICT OF INTEREST

Author declares no conflicting interests.

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