Systems modelling predicts chronic inflammation and genomic instability prevent effective mitochondrial regulation during biological ageing.

Alvaro Martinez Guimera et al

1. Institute for Cell and Molecular Biosciences (ICaMB), Ageing Research Laboratories, Campus for Ageing and Vitality, Newcastle University, United Kingdom

2. Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

The regulation of mitochondrial turnover under conditions of stress occurs partly through the AMPK-NAD-PGC1a-SIRT1 signalling pathway. This pathway can be affected by both genomic instability and chronic inflammation since both of these will result in an increased rate of NAD degradation through PARP1 and CD38 respectively. In this work we develop a computational model of this signalling pathway, calibrating it with- and validating it against- experimental data. The computational model is used to understand how the induction of increased mitochondrial turnover under conditions of stress may be affected by the molecular changes promoted by genomic instability, chronic inflammation and biological ageing in general. We report that the AMPK-NAD-PGC1a-SIRT1 signalling pathway becomes blunted with age and that this can prime for the accumulation of dysfunctional mitochondria. We argue that this is part of a ‘molecular habituation’ phenomenon that occurs during biological ageing where constitutive signals arising from damage accumulation drive an average reduction in network sensitivity and information transmission, as well as an increase in noise, across the cell.

Introduction

Genomic instability and mitochondrial dysfunction are two robust hallmarks of biological ageing (Lopez-Otin et al., 2013, Fakouri et al., 2019). Both of these can influence, prime, constrain and depend on each other as they co-evolve during the ageing process (Fakouri et al., 2019). DNA repair is a key process that determines the extent of genomic instability that develops with age. One of the substrates needed for effective DNA repair is NAD^+. The reason for this is because NAD+ is a substrate in poly-ADP ribosylation (PARylation) reactions. These are mediated by PARP enzymes and are chemical modifications needed for resolving all types of DNA lesions (Wei and Yu, 2016). However, NAD+ is also needed in energy metabolism and energy sensing (Canto et al., 2015). In fact, NAD^+ is part of a mito-nuclear signalling axis linking genomic stability with mitochondrial turnover and the energetic state of the cell (Canto et al., 2015).

The main molecular players in this pathway are AMPK, PGC1α, NAD, SIRT1 and PARP1. Previous work on the interplay between DNA damage and mitochondrial function has found antagonistic effects between PARP1 activity responding to DNA damage levels and mitochondrial turnover responding to deacetylated PGC1a levels (Canto et al., 2015). This suggests that the regulatory circuit mediates dynamic trade-offs between DNA repair activity and other cellular functions.

Further to genomic instability and mitochondrial function being key drivers of the ageing process, the importance of mitonuclear communication is suggested by studies where NAD+ supplementation has robustly increased both lifespan and healthspan in a variety of organisms (Belenky et al., 2007, Balan et al., 2008, Mouchiroud et al., 2013, Cerutti et al., 2014, Guan et al., 2017, Yaku et al., 2018). Conversely, low NAD+ levels have been associated with ageing pathology and mitochondrial dysfunction (Gomes et al., 2013, Zhu et al., 2015, Zhang et al., 2016). Interestingly, a mito-nuclear communication loop mediated by p21 and ROS has also been established to be a driver of cellular senescence (Passos et al., 2010). All of this suggests a key role of mito-nuclear communication in maintaining a balance between nuclear and mitochondrial maintenance with age (Fakouri et al., 2019).

Changes in the NAD^+ -mediated mito-nuclear communication axis have been reported with age (Mendelsohn and Larrick, 2017). Sirtuin activity, NAD levels and AMPK responsiveness tend to decrease with age (Yaku et al., 2018, Camacho-Pereira et al., 2016, Salminen et al., 2016, Mendelsohn and Larrick, 2017) whilst DNA damage tends to increase with age (Freitas and de Magalhaes, 2011).

It is of interest to investigate how the ageing hallmarks of genomic instability and mitochondrial dysfunction may be linked through changes in mito-nuclear communication. In this work we employ a systems modelling approach to explore how age-related alterations may affect the functionality of the NAD^+-mediated mito-nuclear communication axis.

Results

Model development

A computational model of the AMPK-PGC1α-NAD-SIRT1 axis was developed in COPASI (Hoops et al., 2006). This model is based on coupled ordinary differential equations (ODEs) that simulate changes in molecule abundances over time. The time units of the model correspond to hours and the molecule and volume units of the model correspond to arbitrary units (AU).

The development of the model starts with the identification of a core set of interactions within the pathway (illustrated in Figure 1). In this core network, AMPK lies upstream of PGC1α. Upon AMPK activation via phosphorylation, there ensues a rapid PGC1α phosphorylation and a slower PGC1α deacetylation through an increase in NAD levels.

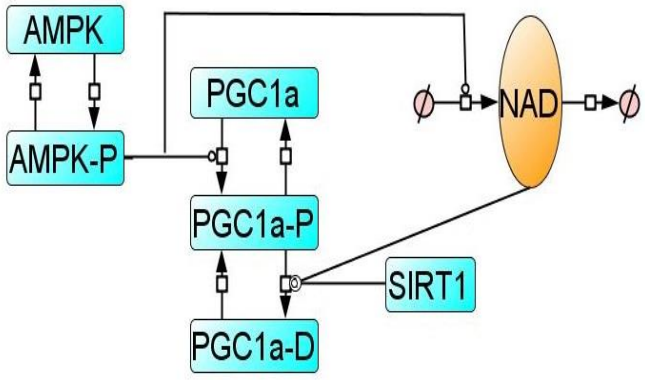

Figure 1. *Network structure delineating the core interactions of the AMPK-PGC1α-NAD-SIRT1 signalling pathway. '-P' suffix denotes a phosphorylated state and '-D' suffix denotes a deacetylated state. The crossed circle denotes an empty state where an entity can be synthesised from or degraded to.*

Not all of the interactions within the core network illustrated in Figure 1 are of the same nature. Interactions denoting (de)phosphorylation and (de)acetylation reactions are mechanistic, since they represent well-defined physicochemical processes. However, the interaction that denotes the increase in NAD levels caused by an increased AMPK activity is phenomenological. That is, it models an underlying process which is more complex than represented in the model. This is since such transition will actually encode many underlying processes such as transcription factor activation, binding to a promoter, mRNA transcription and translation…

Because it is of interest to explore the behaviour of the signalling pathway in response to relevant conditions, the development of the model required the expansion of the core network structure illustrated in Figure 1 to that displayed in Figure 2. The full model expands on the core structure in Figure 1 by firstly introducing the treatment inputs into the network. Of note, the effect of a stimulus is modelled to occur separately to basal rates. For example, NAD degradation is partitioned into PARP1-dependent and PARP1-independent reactions. NAD synthesis is partitioned into AMPK-P – dependent and AMPK-P independent reactions. AMPK dephosphorylation is partitioned into glucose-dependent and glucose-independent reactions. And so on. In addition, instead of using simple transitions to attempt to model phenomenological interactions, 'Delay' variables are introduced. This is in order to faithfully capture the non-

linearity and timescale of phenomenological interactions that are, in reality, more biologically complex than are represented. A 'NegReg' dummy variable is used as a limiter of how much NAD+ may accumulate in the cell (since it is not practical to model the metabolism behind flux shifts that might promote an increased conversion of NAD+ into NADH or other species).

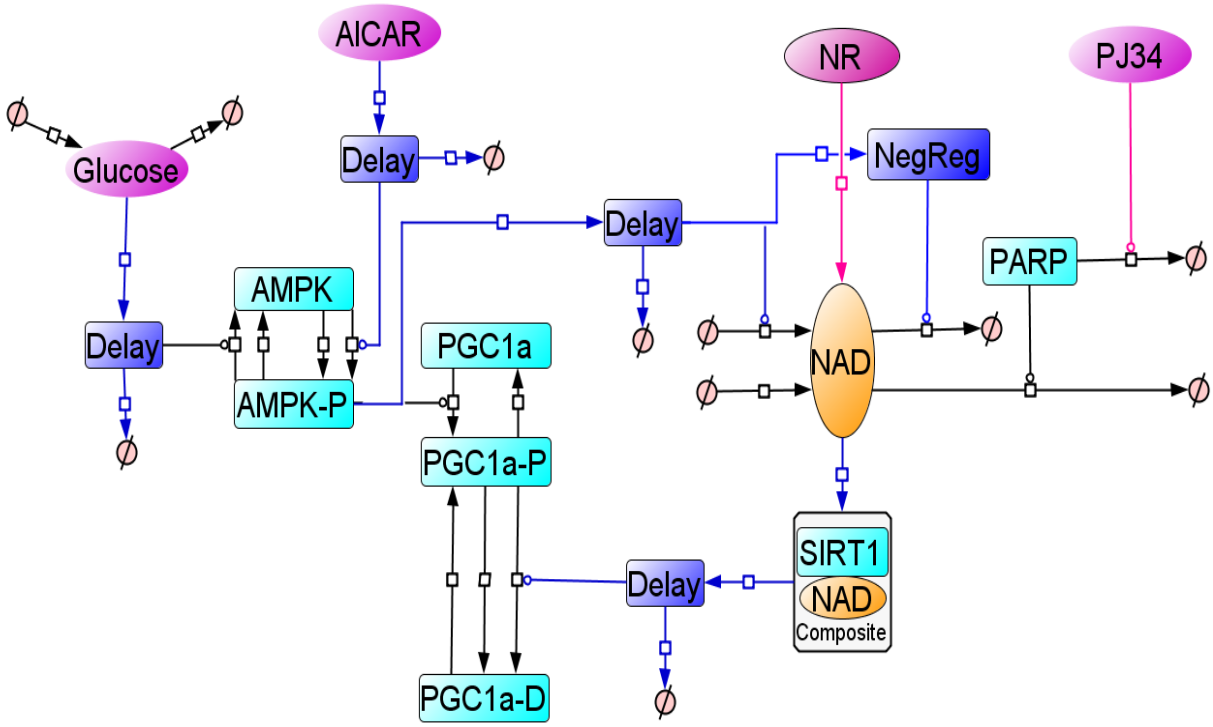

Figure 2. *Model structure of the AMPK- PGC1α-SIRT1-NAD regulatory circuit. '-P' suffix denotes a phosphorylated state and '-D' suffix denotes a deacetylated state. The crossed circle denotes an empty state where an entity can be synthesised from or degraded to. Phenomenological transitions involving "Dummy" variables are shown in dark blue. Phenomenological transitions not involving delay variables are shown in pink. Modelled stimuli (network inputs) are shown in pink. Endogenous biological proteins are shown in light blue with their mechanistic interactions (reactions) shown in black. Note that 'composite' refers to a model species that is the product of two abundances (SIRT1 and NAD) so that the rate of the transition can occur non-linearly through a hill function but still respond to changes in the levels of two species (a hill function can only respond to one species by design).*

Although the model structure is informed by -and devised to approximate- current biological knowledge, the transition kinetics that determine the dynamics of the model structure are based on parameters whose values are largely unknown. However, these can be approximated through the use of parameter estimation procedures. The calibration of the model with experimental data is done through the use of a parameter estimation procedure which explores numerous combinations of potential parameter values to select those which result in the closest fit of the model simulation to the experimental data. Note

that for all network inputs, experimental data from C2C12 myotubes was used for the calibration. See supplementary Figures S1 to S5 for the fits of the model to the calibration data.

To start probing the correspondence of the model with reality, it is necessary to retrospectively test its predictions. This is done by exposing the model to a body experimental data that has not been used in the model calibration processes. The fit between the model simulation and the experimental data will no longer be used to estimate model parameters. It will now be used as an evaluation of how accurately the calibrated model captures the underlying biology. The model validation exercise has involved 22 datasets extracted from 15 peer-reviewed publications (see supplementary Figures S6 to S24). The validations performed indicate that for most network inputs the model results in a good qualitative and quantitative accordance with the experimental data. Indeed, it can be said that the model is, to some degree, an approximation of the underlying biological system of interest. A summary of the depth and breadth of model validation can be seen in supplementary tables 1 and 2. The equations and parameters modelling the AMPK- PGC1α-SIRT1-NAD regulatory circuit can be found in supplementary tables 3-6.

Increased PARP1 activity blunts the responsiveness of the AMPK-NAD-PGC1a-SIRT1 pathway.

To investigate how an energetic stress signal would activate the pathway under conditions of genomic instability, the latter was simulated in the model as a 2.5 fold increase in basal PARP1 activity. The simulation of a 0.5mM AICAR treatment was used a proxy for a stress signal. This signal was introduced into the simulation after the system had equilibrated into a new steady state as a result of the increased genomic instability. As shown in Figure 3, under conditions of genomic instability the activation of the AMPK-NAD-PGC1a-SIRT1 pathway was blunted. The extent of pathway activation as reflected by the absolute levels reached by both NAD and deacetylated PGC1a was reduced. As expected, this pathway blunting occurred at the level of NAD since the upstream activation of AMPK remained unaffected (lines are superimposed in the time course for this species).

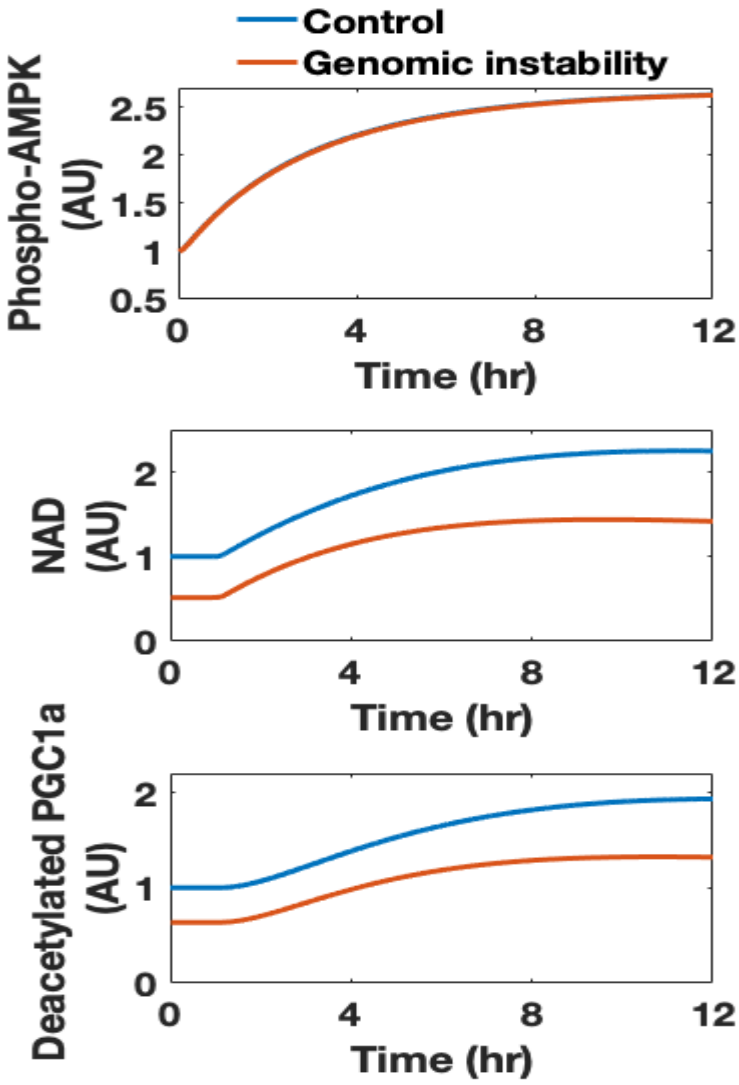

Figure 3. *Simulated time courses of the AMPK-NAD-PGC1a-SIRT1 pathway in response to 0.5mM AICAR treatment under the presence or absence of genomic instability. Note that genomic instability refers to a 2.5 fold increase in PARP1 activity and Phospho-AMPK refers to the PhosphoThr172 – AMPK species.*

Increased NAD degradation limits the accumulation of the NAD signal.

The fact that increased NAD degradation dampens the activation of the signalling pathway is also indicated by the ability of different treatments to rescue the pathway activation under conditions of genomic instability. Model simulations in Figure 4 show that the preconditioning of cells with 0.5mM NR for 24 hrs does not provide a rescue of the pathway activation by 0.5mM AICAR as effective as preconditioning of cells with 1µM PJ34.

This is because the NR supplementation does not target the source of the system dampening: that is an increase in NAD turnover as a result of high PARP1 activity, whilst PJ34 does through the inhibition of PARP1. Model simulations were confirmed by experimental measurements in MEF cells (Figure 5) where genomic instability was introduced though the knockout of Rev1 resulting in a ~2 fold increase in PARP1 levels (supplementary Figure 25).

Note that this data does not mean that NR supplementation should never be as effective as a PJ34 treatment. A high enough NR supplementation could

overwhelm the increased PARP1 activity and activate the pathway by acting as the activation signal itself. Supplementary Figure 26 confirms model simulations (supplementary Figure 27) that show changes in AMPK activation caused by AICAR treatment are not be affected by any condition.

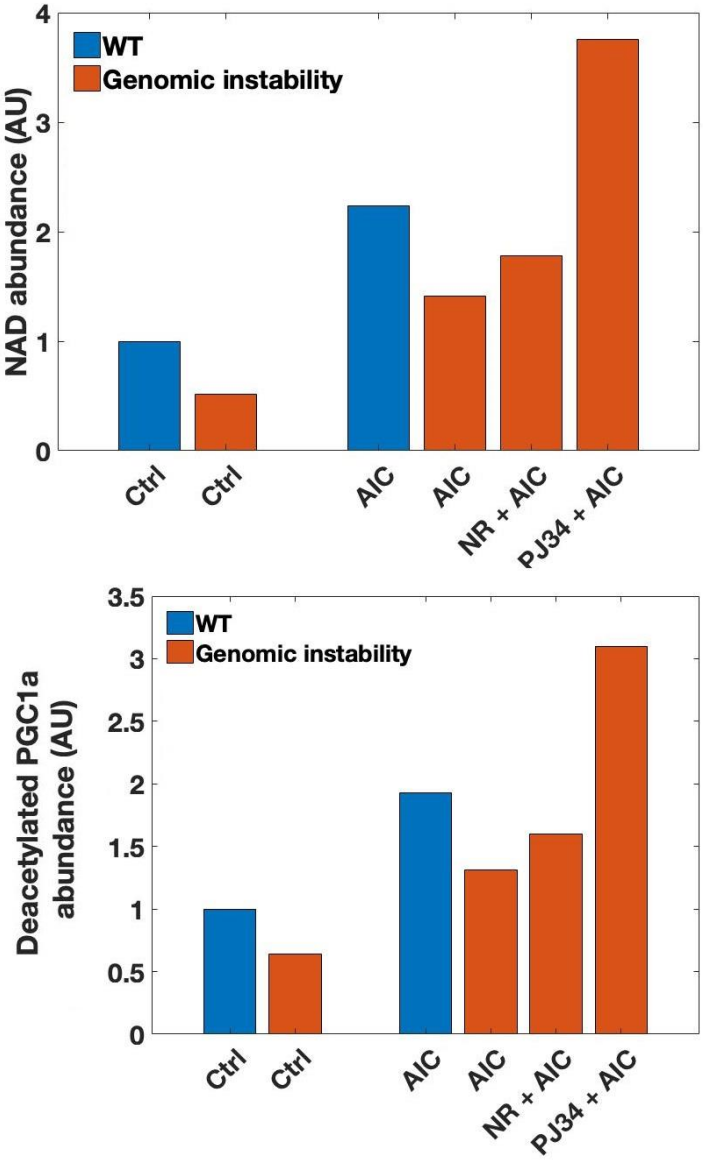

Figure 4. *Model simulation of changes in NAD and deacetylated PGC1α levels as a result of AICAR treatments. AIC=0.5mM AICAR treatment. NR= preconditioning of cells with 0.5mM Nicotinamide Riboside for 24hrs. PJ34 = preconditioning of cells with 1μM PJ34 treatment for 24hrs. The timepoint for AICAR measurement is 12hrs post-treatment initiation. 'Genomic instability' refers to an increase in basal PARP1 activity of 2.5 fold. Ctrl = Control (no AICAR treatment). WT = wildtype.*

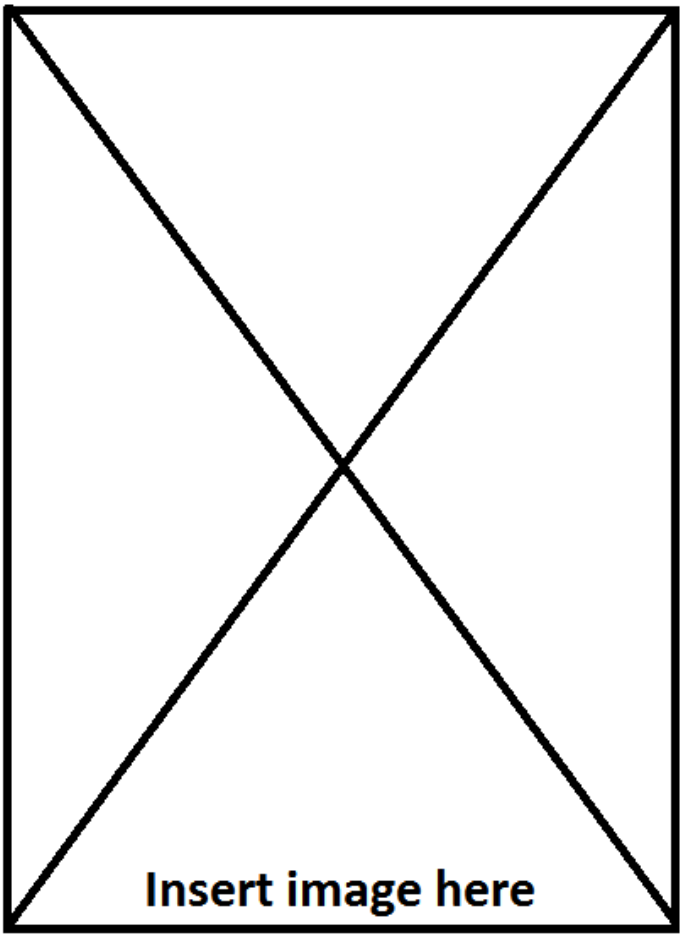

Figure 5. *Measured changes in NAD and deacetylated PGC1α levels as a result of AICAR treatments in Rev1 -/- cells. AIC=0.5mM AICAR treatment. NR= preconditioning of cells with 0.5mM Nicotinamide Riboside for 24hrs. PJ34 = preconditioning of cells with 1μM PJ34 treatment for 24hrs. The timepoint for AICAR measurement is 12hrs post-treatment initiation. Ctrl = Control (no AICAR treatment). WT = wildtype MEF. Error bars correspond to SEM. *=p<0.05.*

This effect is unlikely to be unique to genomic instability.

That an increase in the degradation rate of a “reservoir-molecule” such as NAD will dampen any subsequent attempts to increase its levels through a given signalling-induced flux of the same molecule is likely to be a general phenomenon. Figure 6 shows a simple system of a signalling trigger with flux (k_3) acting on a molecule with synthesis (k_1) and degradation/use (k_2). A signalling event that introduces a given flux of signalling molecule will result in a smaller change in the overall levels of the “reservoir-molecule” under higher turnover conditions (Figure 6b where blue shows low NAD levels and yellow high).

Furthermore, for a given turnover rate of the “reservoir-molecule”, an increase in the degradation rate will result in the dampening of the signal regardless of the magnitude of the signalling flux being introduced (Figure 6c). It can be seen that in this case a decrease in the synthesis rate of NAD (k_1) is not equivalent to an increase in its degradation rate (k_2), since the synthesis rate does not affect the accumulation limit of the signal being introduced (Figure 6c). Hence, any age-related mechanism that results in an increase in the rate of NAD degradation, be it through increased PARP1 or CD38 activity, will have the same dampening effect on the AMPK-NAD-PGC1a-SIRT1 pathway. This means that both genomic instability and chronic inflammation can interfere with the functionality of this pathway during the ageing process.

As an analogy, imagine a fax machine attached to a shredder. The shredder cannot be faster than the fax machine in order for any message to get through. However, the faster the shredder, the less time the message (aka. the signal) will be available. If both the fax machine and the shredder increase their speeds but maintain the same speed-ratio to each other, it would be more difficult to discern any message appearing on the rapidly flowing paper. The message would have to be printed bigger (aka. the signal should be stronger) to maintain a level of discernment. In this sense, a higher turnover confers the system a greater robustness to perturbations.

This perspective sheds some light on how nuclear maintenance may be prioritised over mitochondrial maintenance. An energetic stress signal activates the AMPK-NAD-PGC1α-SIRT1 pathway by increasing SIRT1 activity through the increase in NAD levels but not SIRT1 levels (Canto et al., 2009) – meaning that the reaction is substrate-limited – and so will also increase the substrate for PARP1-mediated reactions. This being whilst a genomic stress signal in the form of increased PARP1 activity will reduce available NAD for SIRT1-mediated reactions. Thus, the prioritisation lies in the asymmetrical influence on the parameters controlling the NAD “reservoir-molecule” as shown in Figure 6. One signal (energetic stress) will influence NAD synthesis and the other (genomic stress) will influence NAD utilisation. Under conditions of conflicting signals where both genomic and energetic stress may be present in an aged cell, genomic maintenance would be prioritised. Note that this mechanism of prioritisation can act in concert to other interactions between PARP1 and SIRT1 (Canto et al., 2015).

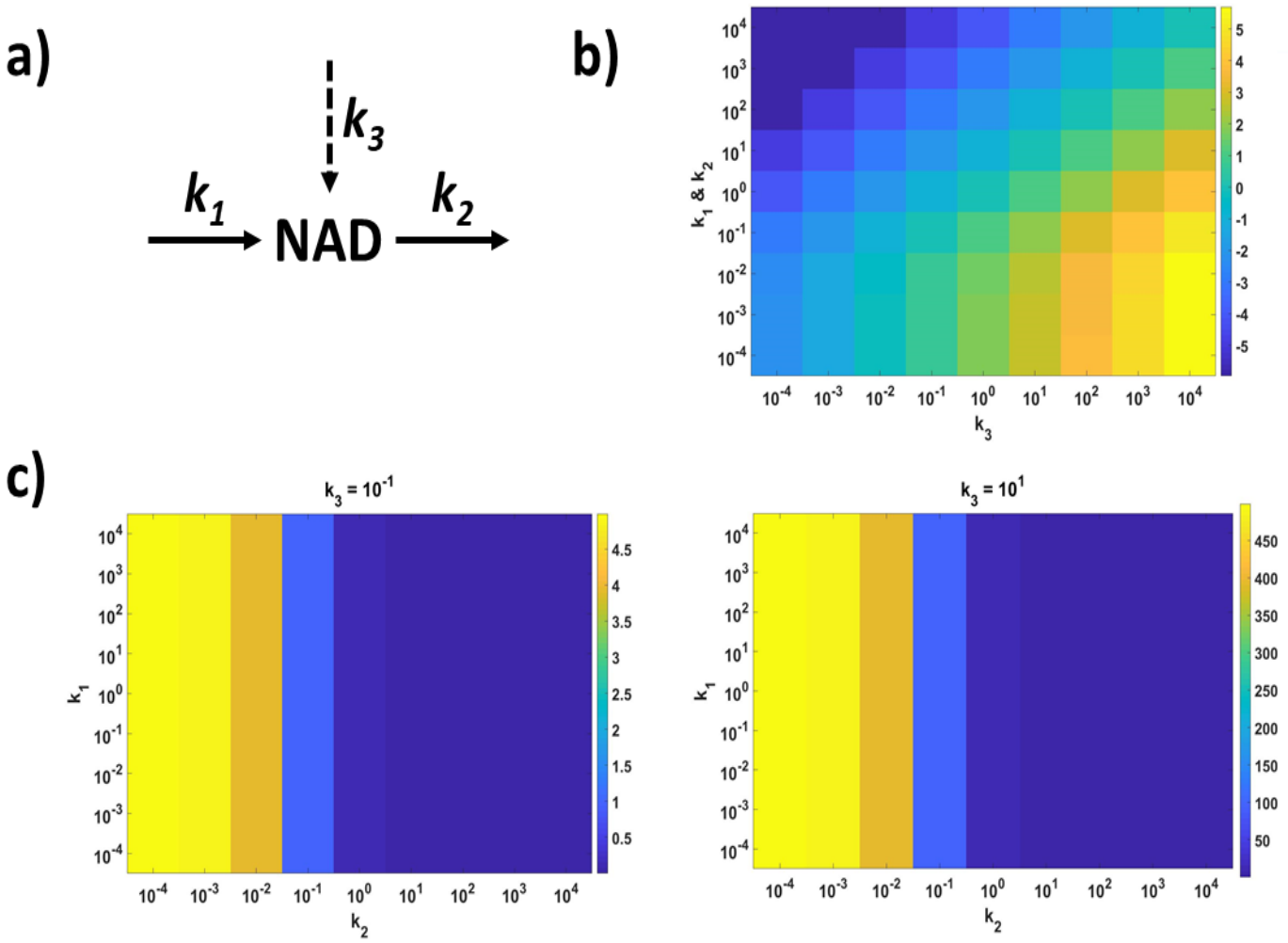

Figure 6. *Peak activation magnitudes of a "reservoir-molecule" under different turnover conditions.* ***a)*** *Model diagram. k_1 = synthesis rate, k_2 = utilisation rate, k_3 =* signalling flux. ***b)*** *Peak activation magnitude (log-transformed) by signalling flux k_3 at different turnover rates (where k_1=k_2).* ***c)*** *Peak activation magnitude for two different k_3 signalling flux values at different combinations of synthesis and utilisation rates (k_1 and k_2).*

Reduced pathway activation promotes the accumulation of dysfunctional mitochondria.

What would be the functional consequence of a dampened AMPK-NAD-PGC1a-SIRT1 signalling pathway? Considering that mitophagy can be triggered through this pathway under conditions of stress (Herzig and Shaw, 2018, Rabinovitch et al., 2017, Zhang et al., 2018) it is feasible that a pathway dampening due to a higher rate of NAD utilisation would lead to a reduced ability to maintain healthy mitochondrial populations through this signalling axis. This would be expected to result in an increased rate of accumulation of dysfunctional mitochondria.

This can be shown in principle by coupling the validated model of the AMPK-NAD-PGC1a-SIRT1 signalling axis to a simple model of mitochondrial populations (see Figure 7 below). In such a model, newly formed (healthy) mitochondria can become damaged/old at a given rate. The latter can then trigger AMPK activation through an increased AMP/ATP ratio in order to enhance mitophagy and remove the excess old mitochondria. Upon pathway activation, deacetylated PGC1α induces mitophagy and thus the disappearance of both types of mitochondrial populations at different rates (in the scale of those reported by Dalle Pezze et al. (2014)). To model the age-dependent accumulation of dysfunctional mitochondria, we introduce a positive feedback loop where mitochondrial dysfunction can prime for more mitochondrial dysfunction.

The simulation of this positive feedback loop results in a given rate of damaged/old mitochondrial accumulation when simulated over 80 years as shown in Figure 8. Note that the simulation of the mitochondrial module alone involves assuming a fixed value of 1 a.u of deacetylated PGC1α where old/damaged mitochondria are not coupled to AMPK activation. See equations and parameter values for the mitochondrial module in supplementary tables 7-10.

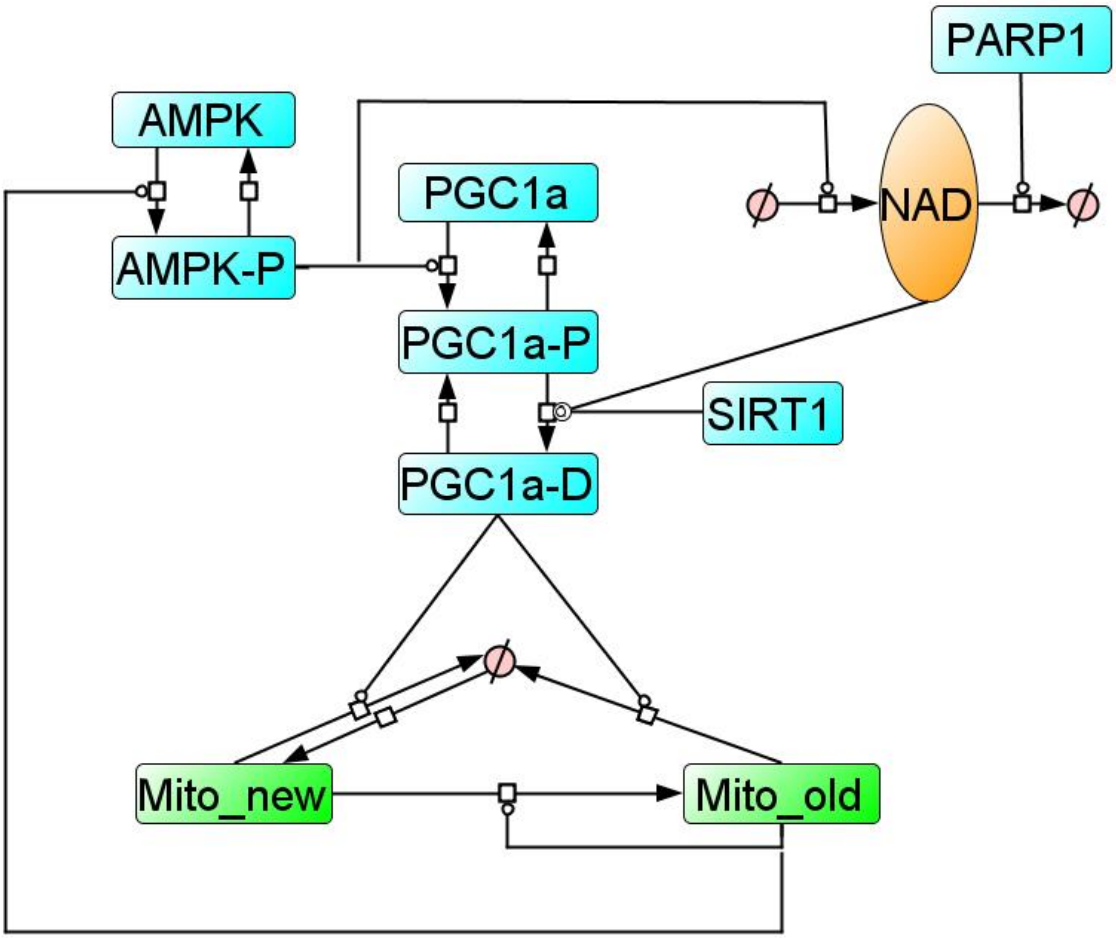

Figure 7. *Coupling of the AMPK-NAD-PGC1a-SIRT1 model (shown in simplified form in blue) with a simple model of mitochondria populations (green). Dashed circle denotes a an 'empty' state where molecules can be synthesised from or utilised/degraded to.*

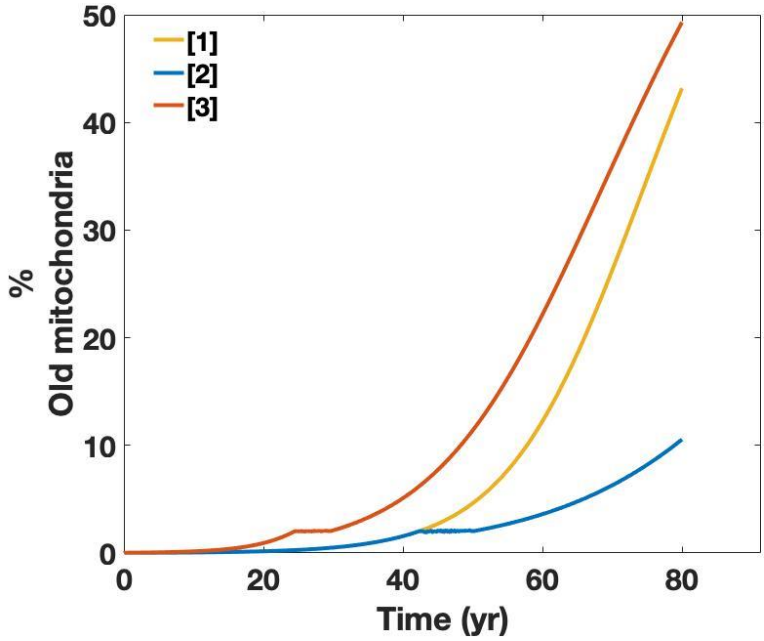

Figure 8. *Accumulation of dysfunctional mitochondria over 80 years simulated by [1] mitochondrial module alone; [2] mitochondrial module coupled to the AMPK-NAD-PGC1α-SIRT1 signalling axis with a fixed PARP1 level of 1 a.u; [3] mitochondrial module coupled to the AMPK-NAD-PGC1α-SIRT1 signalling axis with a fixed PARP1 level of 2.5 a.u.*

Figure 8 shows how the coupling of the mitochondrial module to the AMPK-NAD-PGC1α-SIRT1 axis with a PARP1 value fixed to 1 a.u results in a substantially reduced accumulation of dysfunctional mitochondria over 80 years (Figure 8 curve 1 vs curve 2). This is since the stress conditions associated with the accumulation of dysfunctional mitochondria can now result in increased mitophagy through the activation of the AMPK-NAD-PGC1α-SIRT1 pathway. However, when PARP1 value was fixed to 2.5, the accumulation of dysfunctional mitochondria is even faster than if the mitochondria were not able to be regulated (Figure 8 curve 3 vs curve 1). This is because the high PARP1 activity lowered basal deacetylated PGC1α levels below the value of 1 a.u which is assumed in the simulations involving the mitochondrial module alone. Such simulations would suggest that increased NAD degradation with age, caused by genomic instability, chronic inflammation or both, would interfere with the ability of mitochondria to regulated. This is especially since the AMPK-NAD-PGC1α-SIRT1 pathway also controls FOXO-induced mitophagy through the same mechanism (Canto et al., 2009) and so the pathway dampening would affect mitophagy induction through two main regulatory transcription factors: FOXO and PGC1α.

To make the simulations more realistic, the positive feedback loop driving a given rate of dysfunctional mitochondria accumulation over 80 years can be coupled to PARP1 so that the levels of this molecule gradually increase over the years instead of remaining fixed at a given value. Modelling such age-related changes to PARP1 levels still shows the same effect as that shown in Figure 8 (supplementary Figure 28). Perhaps unsurprisingly, simulating an age-related decrease in SIRT1 levels (supplementary Figure 29) and AMPK levels (supplementary Figure 30) in a similar manner also results in an increased accumulation of dysfunctional mitochondria due to the reduced ability of the latter to activate the AMPK-NAD-PGC1α-SIRT1 signalling pathway to induce mitophagy. Interestingly, an age-related increase in AMPK dephosphorylation

simulated by an age-related hyperglycaemia does not result in any changes to the accumulation of dysfunctional mitochondria since the constant stress signal causing an increased AMPK phosphorylation would balance the increased dephosphorylation (supplementary Figure 31).

In accordance with the established beneficial effects of life-long NAD supplementation, the simulation of this intervention by fixing NR to a value of 100 a.u (to model a life-long supplementation with 0.1mM Nicotinamide Riboside at the cellular level) leads to a marked reduction in the accumulation of dysfunctional mitochondria over 80 years (supplementary Figure 32).

Discussion

NAD is a molecule involved in a myriad of biological processes (Fakouri et al., 2019). Its relevance to the ageing process is highlighted by a multitude of studies that report the modulation of organism lifespan and healthspan through changes in basal NAD levels (Belenky et al., 2007, Balan et al., 2008, Mouchiroud et al., 2013, Cerutti et al., 2014, Guan et al., 2017). The lowering of basal NAD levels with age has been associated with mitochondrial dysfunction (Camacho-Pereira et al., 2016). In this work we demonstrate how the latter can be promoted by an increased turnover of NAD in addition to its absolute levels *per se*. Whilst low NAD levels can be a source of metabolic stress, a high NAD turnover can prevent an adaptation to this stress through increased mitophagy. Interestingly, Dalle Pezze et al. (2014) reported a similar effect where pharmacological interventions aimed at reversing the senescent state of human fibroblast cells were less effective due to a higher mitochondrial turnover.

This is not the only mechanism that can render a regulatory system less sensitive to signalling. We also demonstrate that reported changes to the AMPK-NAD-PGC1a-SIRT1 pathway such as decreased AMPK levels and SIRT1 levels would also result in a loss of pathway sensitivity. The computational model did not imply this for an age-related increase in AMPK dephosphorylation. This is because the model predicts the stress signal that tries to activate AMPK becomes constitutively present and thus balances out an increase in the dephosphorylation of this molecule. However, this assumes that AMPK's ability to sense AMP/ADP and be subsequently phosphorylated remains unchanged with age. This assumption may not always hold true (Hardman et al., 2014).

It thus seems that the AMPK-NAD-PGC1a-SIRT1 pathway can be blunted through various different mechanisms during biological ageing. This observation is not unique to this regulatory circuit, as can be exemplified by mTOR signalling (Francaux et al., 2016, Carroll et al., 2017), Nrf2 (Zhang et al., 2015) and redox signalling in general (Vasilaki et al., 2006, McDonagh et al., 2014,

Cobley et al., 2019) , p38 signalling (Xiao and Majumdar, 2000, Suh and Park, 2001), HSF1 activation (Lu et al., 2000, Heydari et al., 2000) and others (Conconi et al., 1996, Carlson et al., 2008, Haak et al., 2009, Bakondi et al., 2011, Dues et al., 2016) .

All of these signalling systems exist within cells as part of a large molecular interaction network. Such a network can be argued to have the following three properties:

i) Its dysregulation can give rise to constitutive signals.
ii) It is highly interconnected.
iii) It is unlikely to display 'perfect adaptation'.

The rationale following property i) is as follows. Since homeostasis is defined as a property where an internal state can be maintained in spite of perturbations then if homeostasis fails there should be a constitutive elevation or decrease in the level of biological entities involved in the system. These are changes recognisable by baseline measurements. Constitutively elevated or diminished biological entities will be likely to serve as inputs or signals to other cellular pathways through cross-talk. In such a way, stochastic damage imprints as a substantial homeostatic perturbation to the system in the form of a constitutive signal.

Property number ii) suggests that these constitutive signals are unlikely to be contained within signalling pathways. Biological networks are characterised by a high degree of cross-talk between signalling pathways which allows the computing of complex signals and result in rich information processing capabilities (Hormoz, 2013, Kim et al., 2015, Harush and Barzel, 2017). However, such crosstalk between pathways can also be a source of vulnerability since they can spread the dysregulation from one pathway to another (Carlson et al., 2008).

Property number iii) gives an indication on the ability of biological networks to adapt to such constitutive signals when they spread throughout the molecular network through crosstalk. In order for a signalling system to retain a homeostatic state under the presence of a constant signal in the environment it needs to display 'perfect adaptation' (Shankar et al., 2015, Ferrell, 2016). This is a phenomenon where the signalling system is able to reset its sensing due to the strong influence of a negative regulatory molecule that counteracts the constant stimulus from the signal. The problem is that even within simple molecular networks of three molecular elements, where the stimulus affects the most upstream element in the signalling circuit, perfect adaptation to constitutive signals only occurs for very constrained network features (Ma et al., 2009). In the case of a large molecular interaction network, where constitutive signals can affect any given pathway at a point downstream of where the negative regulator might have evolved to act upon, perfect adaptation seems unlikely to occur. It is difficult to envision how a whole cell network-level perfect adaptation might function or evolve. Without perfect adaptation, a constitutive signal in the network results in a change in the homeostatic state of the affected regulatory pathways.

What would be the most likely functional consequence of a constitutive signal being present within a regulatory circuit? Should the signal be inhibitory, the pathway would be rendered less responsive. Should the signal be activatory, the pathway could still lose sensitivity through the submaximal upregulation of negative regulator molecules that are insufficient to provide the system with a 'perfect adaptation' but render the pathway less sensitive to subsequent stimuli (Martinez Guimera et al., 2018). Note that depending on the network properties a constitutive signal could also result in a pathway sensitisation. The presence of constitutive signals in the form of basally elevated network activities have been shown to be a source of noise in a cellular system and to result in an overall loss of sensitivity and information transmission (Voliotis et al., 2014, Martinez Guimera et al., 2018). This is perhaps unsurprising when considering that signalling pathways have evolved to provide a close-to-optimal level of information transmission (Tkacik et al., 2008, Uda and Kuroda, 2016) and thus any change to the underlying parameters of the network is more likely than not to move the system away from the homeostatic optimality and reduce information transmission. Ageing would thus result in a dissipation of the rich patterns of information flow across biological networks (Kim et al., 2015, Harush and Barzel, 2017).

As an example, consider the TGFβ signalling pathway. Should there be an age-related increase in phospho-Erk levels, this would be expected to feed into the TGFβ pathway as a constitutive inhibitory signal that dampens the activation of the pathway (von Bernhardi et al., 2015). Conversely, if phospho-Erk levels decrease with age then the TGFβ pathway could enter a state of low-level basal activation which results in in increased degradation of TGFβ receptors by I-Smad/Smurf as a negative feedback loop (Di Guglielmo et al., 2003, Zi and Klipp, 2007) and consequently a reduced responsiveness of the pathway to TGFβ stimulation.

If a regulatory pathway is less sensitive to a stimulus, then the distribution of abundance values that any given molecule in the pathway may have under the presence of a signal will have a greater overlap with the distribution of values that the same molecule may have under the presence of no signal (see Figure 9). Therefore, a cell is less able to discern whether the signal is in the environment or not. This translates into a greater fraction of cells within a tissue responding inappropriately to a given stimulus (Uda and Kuroda, 2016, Martinez Guimera et al., 2018).

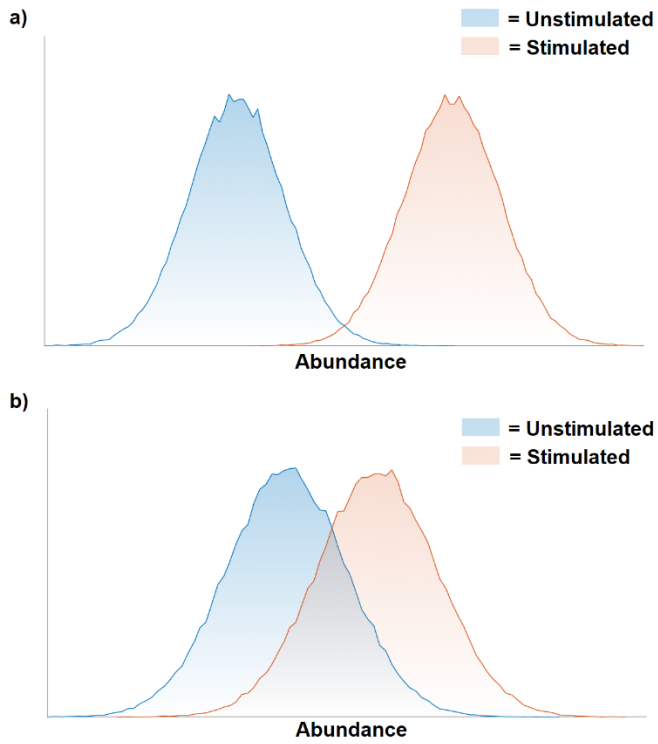

Figure 9. *Cellular discernment of a physiological signal under* ***a)*** *healthy conditions* ***b)*** *aged conditions. A reduction in the change of the mean abundance caused by a stimulus (loss of sensitivity) and an increase in the variance of possible abundances (increase in noise) will result in a greater overlap between the (un)stimulated distributions. Hence, cells are less able to discern the presence of the signal and this translates into a higher proportion of cells within a tissue that respond inappropriately to their environment.*

The sequence of events would thus involve the translation of stochastic cellular damage into permanent imprints into the system, aka. constitutive signals, through mechanisms such a mutation or self-sustaining processes (Mitrophanov and Groisman, 2008, Endo, 2009, Passos et al., 2010, Pratt et al., 2011, Holmes and Diamond, 2012, Zorov et al., 2014). The effect of constitutive signals would then spread throughout the molecular interaction network through crosstalk between regulatory circuits, resulting in an average decrease in pathway sensitivity and information flow and an average increase in system noise.

Heterogeneity in this setting could arise at various points such as the stochasticity of the transient insults (damage) that trigger the constitutive signals, the nature and the number of the constitutive signals and the wiring and parameter values characterising different affected regulatory pathways in different types of cells. Indeed, some pathways might be affected by constitutive signals and some might not. Hence, the phenomenon can only be described to occur on average across the cell's entire molecular network.

In this context a life-extending intervention such as NAD supplementation might provide a local rescue in the network function by alleviating the effects of a higher NAD turnover caused by the higher CD38 and/or PARP1 activities that are actively maintained by constitutive signals arising from chronic inflammation and/or genomic instability respectively. However, there would still be a

significant amount of dysfunctionality being actively maintained by other or the same constitutive signals in different parts of the cellular network.

In physiology, the diminishing of a response under conditions of frequent stimulation is termed ‘habituation’. The described phenomenon can thus be regarded as a whole cell ‘molecular habituation’ effect where ageing is characterised by an average reduction in both cell sensitivity and information transmission and an average increase in network noise. It is worth noting that information in this context represents the ability of a given entity in the cell to tell us –or reduce the uncertainty- about the state of another entity in the network (aka. the mutual information between any two species in the molecular network). Such hallmarks have already been observed in senescent cells and associated with reduced intervention effectiveness (Dalle Pezze et al., 2014). It would be expected that because each cell or cell-type will have stochastic damage translate into different constitutive signals percolating through the network in different ways, that they will have distinct dysregulation ‘*omic*’ signatures.

Conclusion

The increased NAD degradation reported with age is predicted to lead to a dampening of the activation of the AMPK-NAD-PGC1α-SIRT1 signalling pathway. Consequently, mitophagy is less able to be induced under conditions of stress and so dysfunctional mitochondria accumulate. Both genomic instability and chronic inflammation result in an increase in NAD degradation and can be viewed as age-related constitutive signals that interefere with mito-nuclear communication. The nature of this age-related dysregulation can be regarded as a ‘molecular habituation’ phenomenon in the cell’s molecular network. This involves stochastic damage resulting in homeostatic dysregulations which manifest as constitutive signals that percolate through the molecular interaction network stabilising an average state of increased noise, reduced sensitivity and reduced information flow.

Methods

Computational

Model simulation: A computational model of the AMPK-PGC1α-NAD-SIRT1 axis was developed in COPASI (Hoops et al., 2006). This model is based on

coupled ordinary differential equations (ODEs) that simulate changes in species' abundances over time. The model is simulated deterministically using the LSODA algorithm with values of '*Relative Tolerance*' and '*Absolute Tolerance*' of $1e^{-6}$ and $1e^{-12}$ respectively for a '*Max internal steps*' of *10000*.

Parameter estimation: The parameter estimation procedure was carried out using a 'global-chaser' strategy in COPASI (Welsh et al., 2018). The initial round of parameter estimation involved the use of the '*Particle Swarm*' algorithm with an '*Iteration Limit*' setting of *4000* and a '*Swarm size*' of *100* (with '*Std. Deviation*' left at standard setting of $1e^{-6}$). The '*Progress of Fit*' plot in the '*Output assistant*' was activated in order to visually confirm the minimization of the objective function to a stable minimum. If a stable minimum was not achieved then the algorithm '*Iteration Limit*' was increased. Once this requisite was satisfied the model parameters were updated with the estimated ones. The second round of parameter estimation involved the use of the 'Hooke&Jeeves' algorithm under standard settings.

References

BAKONDI, E., CATALGOL, B., BAK, I., JUNG, T., BOZAYKUT, P., BAYRAMICLI, M., OZER, N. K. & GRUNE, T. 2011. Age-related loss of stress-induced nuclear proteasome activation is due to low PARP-1 activity. *Free Radic Biol Med,* 50**,** 86-92.

BALAN, V., MILLER, G. S., KAPLUN, L., BALAN, K., CHONG, Z. Z., LI, F., KAPLUN, A., VANBERKUM, M. F., ARKING, R., FREEMAN, D. C., MAIESE, K. & TZIVION, G. 2008. Life span extension and neuronal cell protection by Drosophila nicotinamidase. *J Biol Chem,* 283**,** 27810-9.

BELENKY, P., RACETTE, F. G., BOGAN, K. L., MCCLURE, J. M., SMITH, J. S. & BRENNER, C. 2007. Nicotinamide riboside promotes Sir2 silencing and extends lifespan via Nrk and Urh1/Pnp1/Meu1 pathways to NAD+. *Cell,* 129**,** 473-84.

CAMACHO-PEREIRA, J., TARRAGO, M. G., CHINI, C. C. S., NIN, V., ESCANDE, C., WARNER, G. M., PURANIK, A. S., SCHOON, R. A., REID, J. M., GALINA, A. & CHINI, E. N. 2016. CD38 Dictates Age-Related NAD Decline and Mitochondrial Dysfunction through an SIRT3-Dependent Mechanism. *Cell Metab,* 23**,** 1127-1139.

CANTO, C., GERHART-HINES, Z., FEIGE, J. N., LAGOUGE, M., NORIEGA, L., MILNE, J. C., ELLIOTT, P. J., PUIGSERVER, P. & AUWERX, J. 2009. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. *Nature,* 458**,** 1056-60.

CANTO, C., MENZIES, K. J. & AUWERX, J. 2015. NAD(+) Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus. *Cell Metab,* 22**,** 31-53.

CARLSON, M. E., SILVA, H. S. & CONBOY, I. M. 2008. Aging of signal transduction pathways, and pathology. *Exp Cell Res,* 314**,** 1951-61.

CARROLL, B., NELSON, G., RABANAL-RUIZ, Y., KUCHERYAVENKO, O., DUNHILL-TURNER, N. A., CHESTERMAN, C. C., ZAHARI, Q., ZHANG, T., CONDUIT, S. E., MITCHELL, C. A., MADDOCKS, O. D. K., LOVAT, P., VON ZGLINICKI, T. & KOROLCHUK, V. I. 2017. Persistent mTORC1 signaling in cell senescence results from defects in amino acid and growth factor sensing. *J Cell Biol,* 216**,** 1949-1957.

CERUTTI, R., PIRINEN, E., LAMPERTI, C., MARCHET, S., SAUVE, A. A., LI, W., LEONI, V., SCHON, E. A., DANTZER, F., AUWERX, J., VISCOMI, C. & ZEVIANI, M. 2014. NAD(+)-dependent activation of Sirt1 corrects the phenotype in a mouse model of mitochondrial disease. *Cell Metab,* 19**,** 1042-9.

COBLEY, J. N., SAKELLARIOU, G. K., HUSI, H. & MCDONAGH, B. 2019. Proteomic strategies to unravel age-related redox signalling defects in skeletal muscle. *Free Radic Biol Med,* 132**,** 24-32.

CONCONI, M., SZWEDA, L. I., LEVINE, R. L., STADTMAN, E. R. & FRIGUET, B. 1996. Age-related decline of rat liver multicatalytic proteinase activity and protection from oxidative inactivation by heat-shock protein 90. *Arch Biochem Biophys,* 331**,** 232-40.

DALLE PEZZE, P., NELSON, G., OTTEN, E. G., KOROLCHUK, V. I., KIRKWOOD, T. B., VON ZGLINICKI, T. & SHANLEY, D. P. 2014. Dynamic modelling of pathways to cellular senescence reveals strategies for targeted interventions. *PLoS Comput Biol,* 10**,** e1003728.

DI GUGLIELMO, G. M., LE ROY, C., GOODFELLOW, A. F. & WRANA, J. L. 2003. Distinct endocytic pathways regulate TGF-beta receptor signalling and turnover. *Nat Cell Biol,* 5**,** 410-21.

DUES, D. J., ANDREWS, E. K., SCHAAR, C. E., BERGSMA, A. L., SENCHUK, M. M. & VAN RAAMSDONK, J. M. 2016. Aging causes decreased resistance to multiple stresses and a failure to activate specific stress response pathways. *Aging (Albany NY),* 8**,** 777-95.

ENDO, M. 2009. Calcium-induced calcium release in skeletal muscle. *Physiol Rev,* 89**,** 1153-76.

FAKOURI, N. B., HOU, Y., DEMAREST, T. G., CHRISTIANSEN, L. S., OKUR, M. N., MOHANTY, J. G., CROTEAU, D. L. & BOHR, V. A. 2019. Toward understanding genomic instability, mitochondrial dysfunction and aging. *FEBS J,* 286**,** 1058-1073.

FERRELL, J. E., JR. 2016. Perfect and Near-Perfect Adaptation in Cell Signaling. *Cell Syst,* 2**,** 62-7.

FRANCAUX, M., DEMEULDER, B., NASLAIN, D., FORTIN, R., LUTZ, O., CATY, G. & DELDICQUE, L. 2016. Aging Reduces the Activation of the mTORC1 Pathway after Resistance Exercise and Protein Intake in Human Skeletal Muscle: Potential Role of REDD1 and Impaired Anabolic Sensitivity. *Nutrients,* 8.

FREITAS, A. A. & DE MAGALHAES, J. P. 2011. A review and appraisal of the DNA damage theory of ageing. *Mutat Res,* 728**,** 12-22.

GOMES, A. P., PRICE, N. L., LING, A. J., MOSLEHI, J. J., MONTGOMERY, M. K., RAJMAN, L., WHITE, J. P., TEODORO, J. S., WRANN, C. D., HUBBARD, B. P., MERCKEN, E. M., PALMEIRA, C. M., DE CABO, R., ROLO, A. P., TURNER, N., BELL, E. L. & SINCLAIR, D. A. 2013. Declining NAD(+) induces a pseudohypoxic state disrupting nuclear-mitochondrial communication during aging. *Cell,* 155**,** 1624-38.

GUAN, Y., WANG, S. R., HUANG, X. Z., XIE, Q. H., XU, Y. Y., SHANG, D. & HAO, C. M. 2017. Nicotinamide Mononucleotide, an NAD(+) Precursor, Rescues Age-Associated Susceptibility to AKI in a Sirtuin 1-Dependent Manner. *J Am Soc Nephrol,* 28**,** 2337-2352.

HAAK, J. L., BUETTNER, G. R., SPITZ, D. R. & KREGEL, K. C. 2009. Aging augments mitochondrial susceptibility to heat stress. *Am J Physiol Regul Integr Comp Physiol,* 296**,** R812-20.

HARDMAN, S. E., HALL, D. E., CABRERA, A. J., HANCOCK, C. R. & THOMSON, D. M. 2014. The effects of age and muscle contraction on AMPK activity and heterotrimer composition. *Exp Gerontol,* 55**,** 120-8.

HARUSH, U. & BARZEL, B. 2017. Dynamic patterns of information flow in complex networks. *Nat Commun,* 8**,** 2181.

HERZIG, S. & SHAW, R. J. 2018. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol,* 19**,** 121-135.

HEYDARI, A. R., YOU, S., TAKAHASHI, R., GUTSMANN-CONRAD, A., SARGE, K. D. & RICHARDSON, A. 2000. Age-related alterations in the activation of heat shock transcription factor 1 in rat hepatocytes. *Exp Cell Res,* 256**,** 83-93.

HOLMES, B. B. & DIAMOND, M. I. 2012. Cellular mechanisms of protein aggregate propagation. *Curr Opin Neurol,* 25**,** 721-6.

HOOPS, S., SAHLE, S., GAUGES, R., LEE, C., PAHLE, J., SIMUS, N., SINGHAL, M., XU, L., MENDES, P. & KUMMER, U. 2006. COPASI--a COmplex PAthway SImulator. *Bioinformatics,* 22**,** 3067-74.

HORMOZ, S. 2013. Cross talk and interference enhance information capacity of a signaling pathway. *Biophys J,* 104**,** 1170-80.

KIM, H., DAVIES, P. & WALKER, S. I. 2015. New scaling relation for information transfer in biological networks. *J R Soc Interface,* 12**,** 20150944.

LOPEZ-OTIN, C., BLASCO, M. A., PARTRIDGE, L., SERRANO, M. & KROEMER, G. 2013. The hallmarks of aging. *Cell,* 153**,** 1194-217.

LU, J., PARK, J. H., LIU, A. Y. & CHEN, K. Y. 2000. Activation of heat shock factor 1 by hyperosmotic or hypo-osmotic stress is drastically attenuated in normal human fibroblasts during senescence. *J Cell Physiol,* 184**,** 183-90.

MA, W., TRUSINA, A., EL-SAMAD, H., LIM, W. A. & TANG, C. 2009. Defining network topologies that can achieve biochemical adaptation. *Cell,* 138**,** 760-73.

MARTINEZ GUIMERA, A., WELSH, C. M., PROCTOR, C. J., MCARDLE, A. & SHANLEY, D. P. 2018. 'Molecular habituation' as a potential mechanism of gradual homeostatic loss with age. *Mech Ageing Dev,* 169**,** 53-62.

MCDONAGH, B., SAKELLARIOU, G. K., SMITH, N. T., BROWNRIDGE, P. & JACKSON, M. J. 2014. Differential cysteine labeling and global label-free proteomics reveals an altered metabolic state in skeletal muscle aging. *J Proteome Res,* 13**,** 5008-21.

MENDELSOHN, A. R. & LARRICK, J. W. 2017. The NAD+/PARP1/SIRT1 Axis in Aging. *Rejuvenation Res,* 20**,** 244-247.

MITROPHANOV, A. Y. & GROISMAN, E. A. 2008. Positive feedback in cellular control systems. *Bioessays,* 30**,** 542-55.

MOUCHIROUD, L., HOUTKOOPER, R. H., MOULLAN, N., KATSYUBA, E., RYU, D., CANTO, C., MOTTIS, A., JO, Y. S., VISWANATHAN, M., SCHOONJANS, K., GUARENTE, L. & AUWERX, J. 2013. The NAD(+)/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling. *Cell,* 154**,** 430-41.

PASSOS, J. F., NELSON, G., WANG, C., RICHTER, T., SIMILLION, C., PROCTOR, C. J., MIWA, S., OLIJSLAGERS, S., HALLINAN, J., WIPAT, A., SARETZKI, G., RUDOLPH, K. L., KIRKWOOD, T. B. & VON ZGLINICKI, T. 2010. Feedback between p21 and reactive oxygen production is necessary for cell senescence. *Mol Syst Biol,* 6**,** 347.

PRATT, D. A., TALLMAN, K. A. & PORTER, N. A. 2011. Free radical oxidation of polyunsaturated lipids: New mechanistic insights and the development of peroxyl radical clocks. *Acc Chem Res,* 44**,** 458-67.

RABINOVITCH, R. C., SAMBORSKA, B., FAUBERT, B., MA, E. H., GRAVEL, S. P., ANDRZEJEWSKI, S., RAISSI, T. C., PAUSE, A., ST-PIERRE, J. & JONES, R. G. 2017. AMPK Maintains Cellular Metabolic Homeostasis through Regulation of Mitochondrial Reactive Oxygen Species. *Cell Rep,* 21**,** 1-9.

SALMINEN, A., KAARNIRANTA, K. & KAUPPINEN, A. 2016. Age-related changes in AMPK activation: Role for AMPK phosphatases and inhibitory phosphorylation by upstream signaling pathways. *Ageing Res Rev,* 28**,** 15-26.

SHANKAR, P., NISHIKAWA, M. & SHIBATA, T. 2015. Adaptive Responses Limited by Intrinsic Noise. *PLoS One,* 10**,** e0136095.

SUH, Y. & PARK, S. C. 2001. Differential activation of mitogen-activated protein kinases by methyl methanesulfonate in the kidney of young and old rats. *Mutat Res,* 497**,** 11-8.

TKACIK, G., CALLAN, C. G., JR. & BIALEK, W. 2008. Information flow and optimization in transcriptional regulation. *Proc Natl Acad Sci U S A,* 105**,** 12265-70.

UDA, S. & KURODA, S. 2016. Analysis of cellular signal transduction from an information theoretic approach. *Semin Cell Dev Biol,* 51**,** 24-31.

VASILAKI, A., MCARDLE, F., IWANEJKO, L. M. & MCARDLE, A. 2006. Adaptive responses of mouse skeletal muscle to contractile activity: The effect of age. *Mech Ageing Dev,* 127**,** 830-9.

VOLIOTIS, M., PERRETT, R. M., MCWILLIAMS, C., MCARDLE, C. A. & BOWSHER, C. G. 2014. Information transfer by leaky, heterogeneous, protein kinase signaling systems. *Proc Natl Acad Sci U S A,* 111**,** E326-33.

VON BERNHARDI, R., CORNEJO, F., PARADA, G. E. & EUGENIN, J. 2015. Role of TGFbeta signaling in the pathogenesis of Alzheimer's disease. *Front Cell Neurosci,* 9**,** 426.

WEI, H. & YU, X. 2016. Functions of PARylation in DNA Damage Repair Pathways. *Genomics Proteomics Bioinformatics,* 14**,** 131-139.

WELSH, C. M., FULLARD, N., PROCTOR, C. J., MARTINEZ-GUIMERA, A., ISFORT, R. J., BASCOM, C. C., TASSEFF, R., PRZYBORSKI, S. A. & SHANLEY, D. P. 2018. PyCoTools: a Python toolbox for COPASI. *Bioinformatics,* 34**,** 3702-3710.

XIAO, Z. Q. & MAJUMDAR, A. P. 2000. Induction of transcriptional activity of AP-1 and NF-kappaB in the gastric mucosa during aging. *Am J Physiol Gastrointest Liver Physiol,* 278**,** G855-65.

YAKU, K., OKABE, K. & NAKAGAWA, T. 2018. NAD metabolism: Implications in aging and longevity. *Ageing Res Rev,* 47**,** 1-17.

ZHANG, H., DAVIES, K. J. A. & FORMAN, H. J. 2015. Oxidative stress response and Nrf2 signaling in aging. *Free Radic Biol Med,* 88**,** 314-336.

ZHANG, H., LIU, B., LI, T., ZHU, Y., LUO, G., JIANG, Y., TANG, F., JIAN, Z. & XIAO, Y. 2018. AMPK activation serves a critical role in mitochondria quality control via modulating mitophagy in the heart under chronic hypoxia. *Int J Mol Med,* 41**,** 69-76.

ZHANG, H., RYU, D., WU, Y., GARIANI, K., WANG, X., LUAN, P., D'AMICO, D., ROPELLE, E. R., LUTOLF, M. P., AEBERSOLD, R., SCHOONJANS, K., MENZIES, K. J. & AUWERX, J. 2016. NAD(+) repletion improves mitochondrial and stem cell function and enhances life span in mice. *Science,* 352**,** 1436-43.

ZHU, X. H., LU, M., LEE, B. Y., UGURBIL, K. & CHEN, W. 2015. In vivo NAD assay reveals the intracellular NAD contents and redox state in healthy human brain and their age dependences. *Proc Natl Acad Sci U S A,* 112**,** 2876-81.

ZI, Z. & KLIPP, E. 2007. Constraint-based modeling and kinetic analysis of the Smad dependent TGF-beta signaling pathway. *PLoS One,* 2**,** e936.

ZOROV, D. B., JUHASZOVA, M. & SOLLOTT, S. J. 2014. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev,* 94**,** 909-50.