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'Molecular habituation' as a potential mechanism of gradual homeostatic loss with age

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ABSTRACT

The ability of reactive oxygen species (ROS) to cause molecular damage has meant that chronic oxidative stress has been mostly studied from the point of view of being a source of toxicity to the cell. However, the known duality of ROS molecules as both damaging agents and cellular redox signals implies another perspective in the study of sustained oxidative stress. This is a perspective of studying oxidative stress as a constitutive signal within the cell. In this work, we adopt a theoretical perspective as an exploratory and explanatory approach to examine how chronic oxidative stress can interfere with signal processing by redox signalling pathways in the cell. We report that constitutive signals can give rise to a 'molecular habituation' effect that can prime for a gradual loss of biological function. This is because a constitutive signal in the environment has the potential to reduce the responsiveness of a signalling pathway through the prolonged activation of negative regulators. Additionally, we demonstrate how this phenomenon is likely to occur in different signalling pathways exposed to persistent signals and furthermore at different levels of biological organisation.

1. Introduction

Whilst reactive oxygen species (ROS) are known to be deleterious and unavoidable products of cellular metabolism, it is apparent that these molecules mediate essential signalling functions within cells (Winterbourn, 2015; Wang and Hai, 2016). Just a few examples of processes mediated by redox signalling include the modulation of insulin signalling (Besse-Patin and Estall, 2014), the stress response (Jiang et al., 2011), cell survival (Trachootham et al., 2008) and tissue regeneration (Sen and Roy, 2008). The elucidation of redox signalling pathways occurred in parallel to the accumulation of evidence that various tissues displayed markers of oxidative stress in various pathologies (Besse-Patin and Estall, 2014; Barbieri and Sestili, 2012; De Marchi et al., 2013; Kim et al., 2015; Sosa et al., 2013; Brioche and Lemoine-Morel, 2016; Lepetsos and Papavassiliou, 2016) and the ageing process (Sanz, 2016; Kirkwood and Kowald, 2012). The established double-edged nature of ROS raises questions as to how cells move from a state of controlled ROS production to a state of oxidative stress.

Oxidative stress is defined as a cellular state involving a mismatch between the abundance of oxidant molecules and the antioxidant capacity of the cell, favouring the former (Sies, 2015). The resulting elevation in the intracellular levels of oxidant can be transient or constitutive (Pickering et al., 2013). Transient (acute) oxidative stress is associated with redox signalling. Constitutive oxidative stress is associated with a prolonged state of elevated oxidant levels. Constitutive or chronic oxidative stress thus involves longer time-scales as is the case in chronic diseases, age-related diseases and the ageing process. Oxidative stress has drawn considerable attention due to the intrinsic reactivity of ROS (Winterbourn, 2015). This chemical property confers these molecules the capacity to cause molecular damage, consequently flagging them as potential causal agents of observed homeostatic disruptions in age and disease.

The perspective of studying oxidative stress as a constitutive signal within the cell has generated some insights: for example, how chronic oxidative stress can become a constant inhibitory signal in calcium

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signalling (Gorlach et al., 2015; Roedding et al., 2013) and T cell activation (Fulop et al., 2014). However, it remains unclear how redox signalling within cells may be affected by sustained oxidative stress. In other words, how will redox signalling pathways respond to an acute ROS signal on top of a constitutively elevated basal oxidant level in the cellular environment. This is of physiological significance, since redox signalling pathways have been shown to become dysfunctional in a variety of tissues in contexts where oxidative stress is also present in the cell (Sohal and Orr, 2012; McDonagh et al., 2014; Cobley et al., 2015; Vasilaki et al., 2006; Claflin et al., 2015; Jackson, 2016; Zhang et al., 2015; Done et al., 2016).

The problem becomes whether the constitutive presence of a signal in the environment affects a signalling pathway's ability to transduce a subsequent acute pulse of the same signal. Whilst the reactivity of reactive oxygen species limits the resolution of current experimental methods (Woolley et al., 2013; Ribou, 2016), very few studies have looked at the effects of long-term exposure of cells to controlled oxidant levels (Covas et al., 2013; Tan et al., 2015; Millonig et al., 2012; Sobotta et al., 2013), with even fewer explicitly examining what effect this exposure would have on a subsequent acute redox signal fed through the system. Work published by Pickering et al. seems to indicate that a chronic exposure of cells to elevated oxidant levels can blunt redox-mediated adaptive responses (Pickering et al., 2013).

Testing all of the potential mechanisms via which chronic oxidative stress could affect physiological redox signalling would be a time-consuming endeavour. In this work, we adopt a theoretical perspective as an exploratory and explanatory approach to examine how chronic oxidative stress can interfere with signal processing by redox signalling pathways in the cell. We report that a constitutive signal in the environment has the ability to reduce the responsiveness of the signalling pathway through the prolonged activation of negative regulators. Additionally, we demonstrate how this phenomenon is likely to occur in different signalling pathways exposed to persistent signals and furthermore at different levels of biological organisation.

2. A rationale for a generic redox model

The major redox stress response pathways in the cell, i.e. NFκB, Nrf2, ASK1, HIF1 and HSF1, reveal conserved topological features. In all pathways cellular stress will interfere with an inhibitor-activator complex (Soga et al., 2012; Hoesel and Schmid, 2013; Tebay et al., 2015; Masoud and Li, 2015; Jiang et al., 2015): IκB − NFκB (Hoesel and Schmid, 2013); Keap1–Nrf2 (Tebay et al., 2015); Thioredoxin1–ASK1 (Soga et al., 2012); VHL − HIF1α (Masoud and Li, 2015) or HSP70/90–HSF1 (Jiang et al., 2015). Oxidant molecules will directly disrupt the inhibition of the activator molecule in the case of the Nrf2 and ASK1 responses, arguably the NFκB response (Oliveira-Marques et al., 2009; Morgan and Liu, 2011) and also the HIF1 response (Nanduri et al., 2015; Chandel et al., 2000). The case of HSF1 differs in that oxidative stress is likely to be sensed indirectly through the abundance of unfolded proteins or the activation of other pathways (Yoo et al., 2014; Swan and Sistonen, 2015). In any case, all pathways have been reported to be activated in response to an oxidant stimulus. It follows from this, that in all pathways the activator molecule must undergo a binding event with, or a modification by, a second molecule to be stabilised and perform a function. These are other ASK1 molecules in case of the ASK1 pathway or co-factors in the case of the Nrf2, NFκB, HIF1, HSF1 transcription factors. Additionally, a stabilising phosphorylation step has been reported for all molecules. Eventually the response must be terminated and these pathways must return to their original state: an activator being actively bound by an inhibitor to form an inactive complex. This requires the complex to be regenerated. Such regeneration occurs through the post-translational modification of the inhibitor, e.g. reduction, or through its *de novo* synthesis.

Based on these observations we defined a core generic redox model (Fig. 1), hereafter referred to as Model 1. Such core model consists of

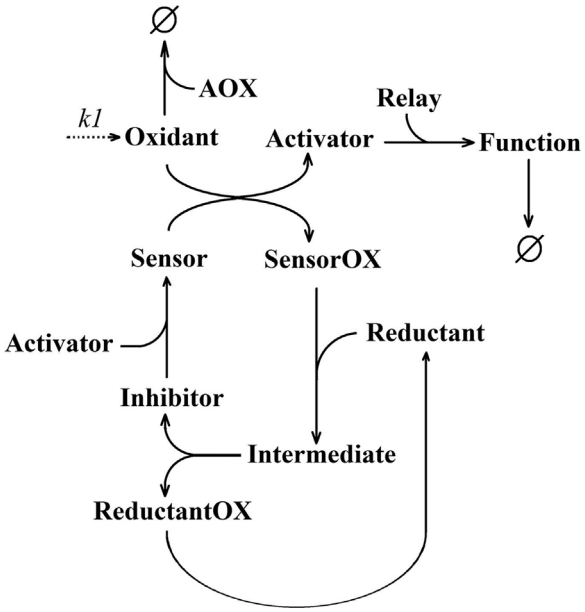

Fig. 1. Network diagram of generic redox signalling Model 1. The steady-state level of the 'Oxidant' signal is modulated through the rate constant k1 which determines the flux of oxidant generation. An oxidation reaction will result in the 'Activator' escaping inhibition and performing a 'Function' after being stabilised by a binding event with a 'Relay' molecule. Meanwhile the inhibitor will undergo a two-step regeneration process before it is able to bind the 'Activator' into an inhibitory complex (Sensor). AOX = Antioxidant. OX suffix = oxidized. Slashed circle = degraded.

'Sensor' molecules that can react with 'Oxidant' molecules which can additionally be scavenged by antioxidant molecules (AOX). Oxidation of 'Sensor' molecules to yield 'SensorOX' will cause the release of an 'Activator' molecule, which must first bind a 'Relay' molecule in order to be stabilised and be able to perform a function. The 'Function' molecule is used as a readout of the activity of the stabilised 'Activator' molecules. Oxidised sensor molecules (SensorOX) can be reduced through a two-step process involving the binding to a 'Reductant' molecule to form an 'Intermediate' complex which is then resolved. The reduced form of the sensor that is not bound to an 'Activator' molecule is termed as an 'Inhibitor' species since it can bind 'Activator' molecules to reform the 'Sensor' complex.

Model 1 ignores feedback mechanisms within the pathways. However, in the major redox stress response pathways outlined above, there are significant uncertainties with regards to the number of negative regulators in each pathway and their relative importance. Despite this, all the pathways have been reported to contain a negative feedback loop occurring through the activator-mediated transcription of inhibitor genes. The negative regulator that mediates the negative feedback loop is able to destabilise the activator in a first step and this results in the subsequent formation of the inhibitor-activator complex. This would correspond to the disruption of the transcriptional complexes and the subsequent nuclear export of NFκB, Nrf2, HSF1 and HIF1 transcription factors or the destabilisation of the ASK1 signalosome. Model 2 is an expansion of Model 1 that includes this element of negative feedback in a simplified time-scale (Fig. 2a). Within Model 2, a negative regulator entity 'NegReg' is introduced into the system downstream of the functional activity of the stabilised activator molecules. This is modelled as 'NegReg' being formed by the reaction between the functional readout molecule (Function) and a second relay molecule (Relay2). Negative feedback occurs by the ability of the 'NegReg' molecules to react with the 'Activator' molecules to render them 'Inactive'.

In the case of the Nrf2 pathway, which is the major regulator of the antioxidant response in cells (Tebay et al., 2015), the main mechanism behind the response shutdown occurs through the delayed activation of