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Identifying the conditions necessary for the thioredoxin ultrasensitive response☆

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Summary Thioredoxin, glutaredoxin, and peroxiredoxin systems (collectively called redoxins) play critical roles in a large number of redox-sensitive cellular processes. These systems are linked to each other by coupled redox cycles and by common reaction intermediates into a larger network.

Previous results from a realistic computational model of the *Escherichia coli* thioredoxin system developed in our group have revealed several modes of kinetic regulation in the system. Amongst others, the coupling of the thioredoxin and peroxiredoxin redox cycles was shown to exhibit the potential for ultrasensitive changes in the thioredoxin concentration and the flux through other thioredoxin-dependent processes in response to changes in the thioredoxin reductase level. Here, we analyse the basis for this ultrasensitive response using kinetic modelling and metabolic control analysis and derive quantitative conditions that must be fulfilled for ultrasensitivity to occur.

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Introduction

Thioredoxins, glutaredoxins and peroxiredoxins (collectively termed redoxins) are present in all living organisms and regulate a diverse array of redox-sensitive processes including DNA synthesis, transcription factor activation, anti-oxidant defence (Toledano et al., 2007) and signal transduction (Finkel, 2011; Winterbourn and Hampton, 2008). In these reactions, redoxins are oxidized by their targets and are therefore coupled to additional redox cycles forming the thioredoxin, glutaredoxin and peroxiredoxin systems, which are linked into an integrated network (Pillay et al., 2013).

This network is complex both in terms of its size and interconnectivity and dysregulation of the network is associated with a number of diseases including cancer, HIV susceptibility, heart disease and several neurodegenerative disorders (Holmgren and Lu, 2010). Further, pathogen redoxin systems are essential for survival in infectious diseases such as tuberculosis and malaria and these systems are consequently being evaluated as drug targets (Jaeger and Flohé, 2006). Computational systems biology approaches could play an important role in precisely delineating roles played by redoxin networks in these pathologies and in identifying druggable targets (Pillay et al., 2013). However, progress here has been limited by the conflicting descriptions of redoxins in the literature and inconsistencies in the quantitative measures and kinetic models of redoxin activity (Pillay et al., 2009, 2013).

Our recent work has resolved several of these contradictions. Using kinetic modelling, we showed that the enzyme-like behaviours (e.g. substrate saturation) attributed to thioredoxins resulted from redistribution of oxidised and reduced thioredoxin in the thioredoxin cycle, and are not due to enzymatic properties of thioredoxin itself (Pillay et al., 2009). Moreover, we described how redoxin system dynamics can result in regulatory designs that cannot be anticipated by studying system components in isolation (Mashamaite et al., 2015).

The insights from our work resulted in the first *in vivo* models of redoxin networks by us and other groups (Adimora et al., 2010; Benfeitas et al., 2014; Pillay et al., 2011). We developed the first realistic model of the *Escherichia coli* thioredoxin system (see Fig. 1). In contrast to the view that the thioredoxin network was analogous to an electrical circuit, we showed this system was interconnected, displayed adaptability and described a novel form of ultrasensitivity (Pillay et al., 2011).

The main result from our previous study was that the reduced thioredoxin concentration (TrxSH), and as a consequence the flux through certain TrxSH-dependent reactions, could respond in an ultrasensitive manner to changes in the levels of thioredoxin reductase (Fig. 2). Here, ultrasensitivity is defined as a slope of greater than one when the response is plotted against the input in double-logarithmic space.

Ultrasensitivity is normally associated with signalling and together with other findings (Finkel, 2011), it is emerging that reactive oxygen species, particularly hydrogen peroxide, are important cellular secondary messengers in signalling, even under normoxic conditions (Winterbourn and Hampton, 2008). However, the quantitative signal properties, targets and dynamics of this process are not clear and our understanding of the balance between redox signalling and redox stress is vastly incomplete. In this paper, we use kinetic modelling and metabolic control analysis to elucidate the mechanistic basis for the ultrasensitive response shown in Fig. 2.

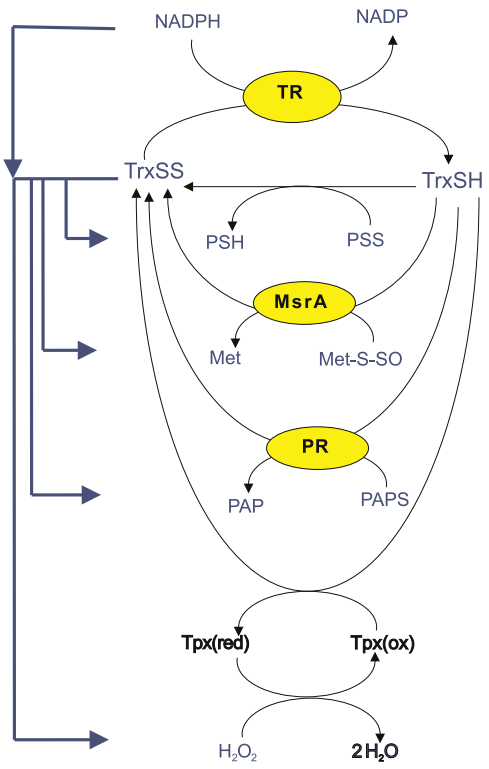

Figure 1 Reaction scheme of the kinetic model of the *E. coli* thioredoxin system. Blue arrows denote electron flow. Abbreviations: TrxSS and TrxSH, oxidised and reduced thioredoxin respectively; TR, thioredoxin reductase; PSS, oxidised protein, Met-S-SO, methionine sulphoxide; MsrA, methionine sulphoxide reductase; PAPS, phosphoadenosine phosphosulphate; PR, PAPS reductase; Tpx, peroxiredoxin. Taken from Pillay et al. (2011).

Methods

Metabolic control analysis

Metabolic control analysis (MCA) is a framework for quantifying the control properties of a steady-state metabolic system in terms of the responses of the system fluxes and metabolite concentrations to perturbations in the rates of the reactions (Kacser et al., 1995; Heinrich and Rapoport, 1974). For this purpose, MCA defines two types of coefficients:

- An *elasticity coefficient* describes the sensitivity of an individual reaction rate towards a change in any concentration x of a substrate, product, or effector that affects the reaction directly. It is defined as the ratio of