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Review

Regulation of the human thioredoxin gene promoter and its key substrates: A study of functional and putative regulatory elements

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ABSTRACT

Background: The thioredoxin system maintains redox balance through the action of thioredoxin and thioredoxin reductase. Thioredoxin regulates the activity of various substrates, including those that function to counteract cellular oxidative stress. These include the peroxiredoxins, methionine sulfoxide reductase A and specific transcription factors. Of particular relevance is Redox Factor-1, which in turn activates other redox-regulated transcription factors.

Scope of review: Experimentally defined transcription factor binding sites in the human thioredoxin and thioredoxin reductase gene promoters together with promoters of the major thioredoxin system substrates involved in regulating cellular redox status are discussed. An *in silico* approach was used to identify potential putative binding sites for these transcription factors in all of these promoters.

Major conclusions: Our analysis reveals that many redox gene promoters contain the same transcription factor binding sites. Several of these transcription factors are in turn redox regulated. The ARE is present in several of these promoters and is bound by Nrf2 during various oxidative stress stimuli to upregulate gene expression. Other transcription factors also bind to these promoters during the same oxidative stress stimuli, with this redundancy supporting the importance of the antioxidant response. Putative transcription factor sites were identified *in silico*, which in combination with specific regulatory knowledge for that gene promoter may inform future experiments.

General significance: Redox proteins are involved in many cellular signalling pathways and aberrant expression can lead to disease or other pathological conditions. Therefore understanding how their expression is regulated is relevant for developing therapeutic agents that target these pathways.

1. Introduction

The thioredoxin (Trx) system is one of the most important antioxidant systems in the cytoplasm of a cell, with a corresponding system in the mitochondria. The cytoplasmic system consists of Trx, which is a 12 kDa redox active protein, and thioredoxin reductase (TrxR), a selenoprotein [1]. The active site of Trx contains two active cysteine residues that undergo reversible oxidation to form a disulfide bond, during the process of transferring reducing equivalents to a disulfide substrate. TrxR then recycles the oxidised Trx protein to a reduced state using NADPH as the electron donor [2]. To date TrxR is the only known enzyme capable of reducing Trx and thus it is an essential component of the Trx system. There are Trx systems in both the cytoplasm and mitochondria. The cytoplasmic system contains Trx-1 and TrxR1, while the corresponding Trx system in the mitochondria is comprised of Trx-2 and TrxR2. For the purposes of this review the terms Trx and TrxR will refer to Trx-1 and TrxR1. Through a reversible redox reaction involving TrxR, Trx can regulate the activity of specific protein substrates in many pathways, including transcription factors [3], apoptotic signalling [4], ribonucleotide reductase [5] and members of the peroxiredoxin family, which degrade hydrogen peroxide [6]. Misregulation of Trx and its substrates can lead to many pathological conditions, including cancer [7,8], and thus their potential transcriptional coregulation is of great interest and relevance to the design of therapeutic agents.

Key transcriptional regulators of the human Trx gene promoter have been identified [9–13]. However it is not clear if the Trx gene is

Abbreviations: AP-1, Activator protein-1; APE, Apurinic/apyrimidinic endonuclease; ARE, Antioxidant response element; ChIP, Chromatin immunoprecipitation; CRE, cAMP response element; CREB, CRE binding protein; Egr-1, Early growth response factor-1; EMSA, Electrophoretic mobility shift assay; Ets, E26 transformation specific; FOXO, Forkhead O; GSH, Glutathione; HIF, Hypoxia inducible factor; Keap1, Kelch-like ECH-associated protein 1; NF-κB, Nuclear Factor-κB; Nrf2, Nuclear factor-erythroid 2 p45-related factor 2; MiTF, Microphthalmia associated transcription factor; MRE, Metal response element; MSR, Methionine sulfoxide reductase; NADPH, Nicotinamide adenine dinucleotide phosphate; NGF, Nerve growth factor; Oct-1, Octamer binding protein; p53, Tumour protein 53; PRDX, Peroxiredoxin; PPAR, Peroxisome proliferator-activated receptor; PPRE, PPAR response element; RA, Retinoic acid; RAR, Retinoic acid receptor; RARE, RA response element; RXR, Retinoid X receptor; Ref-1, Redox Factor-1; ROS, Reactive oxygen species; Sp1, Specificity protein 1; tBHQ, *tert*-Butylhydroquinone; Trx, Thioredoxin-1; TrxR, Thioredoxin reductase 1; TSS, Transcription start site

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coregulated with genes that encode the key substrates for Trx needed for an effective cellular response against oxidative stress. Therefore we selected the human cytoplasmic Trx system genes (Trx and TrxR) and a number of key substrates including all of the peroxiredoxins, methionine sulfoxide reductase A (MSRA), and Redox Factor-1 (Ref-1) to review current knowledge regarding which transcription factor binding sites have been experimentally validated to regulate these gene promoters. We then searched the promoter sequences for other putative transcription factor binding sites.

Peroxiredoxins (PRDXs) are 20–30 kDa thiol-specific antioxidant proteins that scavenge reactive oxygen species (ROS) and form part of the cellular response to oxidative stress [6]. Currently six human PRDXs (PRDX1–6) have been described that degrade H_2O_2 and other peroxides using the thiol groups of their cysteines as the catalytic site. PRDX1–4 contain two conserved cysteines in their active site and utilise Trx as their reductant [6]. PRDX5 is also a substrate for Trx [14] but is classified as an atypical 2-cys PRDX that prefers to reduce alkyl hydroperoxides and peroxynitrite [15]. PRDX6 contains a single conserved cysteine and unlike the other PRDXs utilises GSH but not Trx to catalyse the reduction of H_2O_2 [16,17]. It is included as part of this study to compare its promoter structure to those PRDXs that are regulated by Trx. PRDX1, PRDX2 and PRDX6 are located in the cytoplasm [6], PRDX3 contains a mitochondrial targeting sequence [18] and PRDX4 is secreted outside the cell [19]. PRDX5 is found in the cytoplasm, mitochondria and peroxisomes [20,21].

Another substrate for Trx is MSRA, which reduces oxidised methionine residues [22]. When methionine is oxidised, it can form two different stereoisomers; the Met-S-SO form, which is reduced by MSRA and the Met-R-SO form, which is reduced by MSRB [23]. This oxidation forms an internal disulfide bond in the MSRA protein that is subsequently reduced by the Trx system [24]. The gene for MSRA contains two transcription start sites (TSSs) located 40 kb apart. Therefore two distinct promoters regulate the expression of MSRA: the 'upstream' promoter regulates MSRA1 transcription and the 'downstream' promoter regulates MSRA2 and MSRA3 expression. Products encoded from MSRA1 are located in the mitochondria and those from MSRA2 and MSRA3 are localised in the cytoplasm and nucleus, respectively [25,26]. MSR activity appears to decrease during ageing [27,28], but as yet the regulatory mechanisms are undefined. In addition, a reduced level of MSRA is associated with various neurodegenerative disorders including Alzheimer's disease [29] and Parkinson's disease [30]. Since MSRA is a Trx substrate and is associated with these oxidative stress linked pathological conditions MSRA was selected for inclusion in this current review.

Redox Factor 1 (Ref-1) is a 36.5 kDa protein also known as apurinic/apyrimidinic endonuclease (APE1) and was first described as an important enzyme in the base excision repair (BER) pathway. This pathway is activated to repair apurinic/apyrimidinic (AP) sites, which often occur when ROS causes DNA damage [31]. APE1 catalyses the second step of the pathway, which is to cleave the phosphodiester backbone after the removal of damaged bases by glycosidases in step 1. The cleavage occurs immediately 5′ to an AP site and creates an abasic 5′-deoxyribose phosphate and a 3′ hydroxyl, which is utilised by DNA polymerase β and DNA ligase to continue the repair [31]. Ref-1 also has a redox activity that resides in a separate N-terminal domain of the protein [32]. Ref-1 activity has been shown to enhance the DNA binding ability of transcription factors such as nuclear factor-kappa B NF-κB [33,34], tumour protein 53 (p53) [35,36], hypoxia inducible factor-1α (HIF-1α) [37,38] and activator protein-1 (AP-1) [33,39]. Trx has been shown to bind to Ref-1 *in vitro* and through mammalian two–hybrid assays in Cos-7 cells [40]. Other studies have further implicated Trx as cooperating with Ref-1 within the nucleus of mammalian cells to enhance AP-1 activity [3,41], although there have been surprisingly few studies reported that focus on the physiological relevance of the Trx–Ref-1 interaction. More recently, Ref-1 was shown to have a redox chaperone activity, independent of its own redox activity, whereby it facilitates the reduction of transcription factors by other reducing agents, including Trx [33].

This report focuses on Trx, TrxR, and their major substrates, the PRDX family, MSRA and Ref-1, as they are significant contributors to the antioxidant response and there are potential redundancies or cross regulation between several of these proteins. Since Trx and many of these substrates are upregulated in tumours, or involved in other pathologies, it is possible that a coordinated approach to target their gene expression regulation may present a more effective therapeutic intervention. However we first need to know which transcription factors are involved in antioxidant promoter regulation in response to a specific stimulus. We will discuss the experimental evidence that supports the binding of specific transcription factors to specific sites in the gene promoters and then identify potential binding sites using *in silico* searches. While not all of the putative binding sites will be functional, the potential sites found in these searches may guide the design of future specific functional studies in combination with current knowledge regarding regulation of these gene promoters.

2. Methodology

Promoter regions of eleven human genes that express redox control proteins were selected for this study. They include Trx, TrxR, MSRA1, MSRA2, PRDX1, PRDX2, PRDX3, PRDX4, PRDX5, PRDX6, and Ref-1. All nucleotide sequences were obtained from the NCBI and ENSEMBL databases (Table 1) and cover from −1100 to +200 bp relative to the main TSS. Experimentally mapped TSSs reported in the literature were used where available, otherwise TSSs were assigned using the NCBI and ENSEMBL databases to compare sequenced transcripts to genomic sequence. Consensus transcription factor binding sites in their promoter regions were identified utilising the MatInspector programme [42] of the Genomatix web site (www.genomatix.de), with the Ci-value set to 80%. In particular we focussed on the following transcription factor binding sites, listing first the most studied sites in these gene promoters with respect to oxidative stress: antioxidant response element (ARE), Ets binding site, forkhead element, AP-1, E-box, retinoic acid receptor element (RARE), PPAR response element (PPRE), cAMP response element (CRE), TATA box, specificity protein 1 (Sp1), octamer binding protein (Oct-1) NF-κB, binding site, early growth response factor-1 (Egr-1) sites and the metal response element (MRE). These putative transcription factor-binding sites were cross-referenced with the experimentally validated sites described in the literature.

3. The antioxidant response element (ARE)

Oxidative stress can result from many stimuli or cellular pathologies leading to the activation of different signalling pathways. Consequently, different transcription factors are activated, which bind to quite specific DNA binding elements in gene promoters. The most common DNA element associated with cellular oxidative stress is the antioxidant response element (ARE). This element is found in many phase II detoxifying gene promoters and gene expression is regulated by the binding of nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) [43,44]. In unstimulated cells an inhibitor called Kelch-like ECH-associated protein 1 (Keap1) binds to Nrf2 and targets it for degradation *via* the ubiquitination pathway [45]. During oxidative stress Keap1 is modified such that it no longer interacts with Nrf2, allowing Nrf2 to move into the nucleus [46]. Nrf2 has been shown to bind to an ARE in four promoters out of the eleven, Trx, TrxR, PRDX1 and PRDX6. The reported sites are listed in Tables 2a and 2b and depicted in Fig. 1.

3.1. The Trx gene promoter

Trx, as expected, is induced in response to various oxidative stress stimuli and a potential ARE was first described in the Trx gene promoter in 2001 [10]. Hemin, which is an oxidised form of heme, was utilised as