Published in final edited form as:
Free Radic Biol Med. 2014 February ; 67: 69–80. doi:10.1016/j.freeradbiomed.2013.10.806.

Poly(ADP-ribose) polymerase-1 Modulates Nrf2-dependent Transcription

Tongde Wu[1], **Xiao-Jun Wang**[1], **Wang Tian**[1], **Melba C. Jaramillo**[1], **Alexandria Lau**[1], and **Donna D. Zhang**[1,2]

[1]Department of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, 1703 East Mabel Street, Tucson, AZ, 85721

[2]Arizona Cancer Center, University of Arizona, 1515 North Campbell Avenue, Tucson, AZ 85724, USA

Abstract

The basic leucine zipper (bZIP) transcription factor Nrf2 has emerged as a master regulator of intracellular redox homeostasis by controlling the expression of a battery of redox balancing antioxidants and phase II detoxification enzymes. Under oxidative stress conditions, Nrf2 is induced at the protein level through redox-sensitive modifications on critical cysteine residues in Keap1, a component of an E3 ubiquitin ligase complex that targets Nrf2 for proteasomal degradation. Poly(ADP-ribose) polymerase-1 (PARP-1) is historically known to function in DNA damage detection and repair; however, recently PARP-1 has been shown to play an important role in other biochemical activities, such as DNA methylation and imprinting, insulator activity, chromosome organization and transcriptional regulation. The exact role of PARP-1 in transcription modulation and the underlying mechanisms remain poorly defined. In this study, we report that PARP-1 forms complexes with the antioxidant response element (ARE) within the promoter region of Nrf2 target genes and upregulates the transcriptional activity of Nrf2. Interestingly, PARP-1 neither physically interacts with Nrf2 nor does it promote the expression of Nrf2. In addition, PARP-1 does not target Nrf2 for poly(ADP-ribosyl)ation. Instead, PARP-1 interacts directly with small Maf proteins and the ARE of Nrf2 target genes, which augments ARE-specific DNA-binding of Nrf2, and enhances the transcription of Nrf2 target genes. Collectively, these results suggest that PARP-1 serves as a transcriptional coactivator, upregulating the transcriptional activity of Nrf2 by enhancing the interaction among Nrf2, MafG and the ARE.

Keywords

PARP-1; Nrf2; ARE; Maf; transcriptional co-activator

To whom correspondence should be addressed: Donna D. Zhang, Department of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, 1703 E. Mabel St., Tucson AZ, USA, Tel.: (520) 626-9918; Fax: (520) 626-2466; dzhang@pharmacy.arizona.edu.

Introduction

Nrf2 is a member of the NF-E2 family of transcription factors that plays a key role in the regulation of intracellular redox homeostasis. Under unstressed conditions, Nrf2 is consistently recruited by Kelch-like ECH-associated protein 1 (Keap1) into the Cul3/Rbx1/Keap1 E3 ubiquitin ligase complex to be ubiquitinated and shuttled to the 26S proteasome for degradation. Induction of Nrf2 signaling by chemopreventive compounds or oxidative stress leads to the disruption of the interaction between Keap1 and Nrf2, resulting in stabilization of Nrf2. Accumulated Nrf2 translocates into the nucleus where it forms a heterodimer with a small Maf protein (MafG, MafF or MafK) and binds to the antioxidant response elements (AREs) in the promoters of its target genes. Products of these genes include: (1) intracellular redox-balancing proteins (e.g. NQO1 NAD(P)H quinone oxidoreductase 1 <NQO1>, heme oxygenase-1 <HO-1>, and aldo-keto reductase family 1, member C1<AKR1C1>); (2) phase II detoxifying enzymes (e.g. glutathione S-transferase <GST>, and UDP-glucuronosyltransferase <UGT>, and the catalytic and modifier subunit of glutamate cysteine ligase <GCLC, GCLM>; and (3) transporters (e.g. Multidrug resistance-associated proteins <MRPs>) [1–7].

Nrf2 is mainly regulated by Keap1 at the protein level through proteasomal degradation. Previous studies, however, have also revealed that there are other co-factors that modulate the transcriptional activity of Nrf2. For example, during early hepatocarcinogenesis, monocytic leukemia zinc-finger protein (MOZ) directly binds to MafK and acts as a co-activator of the Nrf2–MafK heterodimer, and consequently induces the expression of the placental form of GST (GSTP), which is elevated during chemical induced hepatocarcinogenesis [8]. Another study done by Shenvi et al. showed that, with the process of aging, binding of Nrf2 to the ARE of the GCLC decreases in the presence of Bach1, and in the absence of CREB-binding protein (CBP) [9]. These studies suggest that under certain conditions, co-factors may be required for fine tuning of the transcriptional activation of Nrf2 target genes.

Poly(ADP-ribose) polymerase-1 (PARP-1) is historically known for its activity to attach the ADP-ribose polymer chains to target proteins, a process known as PARylation and facilitate the process of DNA repair. However, a growing body of evidence suggests that PARP-1 can go beyond DNA repair and is involved in a wide range of biological processes, such as DNA methylation, transcriptional regulation, and cell death [10]. Recent studies have highlighted the complex role of PARP-1 in transcriptional regulation and provided new insights about how PARP-1 plays a role in signal transduction in the nucleus [11]. Four distinct modes of PARP-1-dependent transcriptional regulation have been proposed: (1) As a modulator of chromatin structure by binding to nucleosomes, modifying histone proteins, or regulating the composition of chromatin. For example, by competing with histone H1 for nucleosome binding or by PARylation, PARP-1 excludes H1 from the promoters of some PARP-1-regulated genes and activates gene transcription [12, 13]. (2) As a promoter-specific co-activator/repressor for a variety of transcription factors. Transcription factors usually recruit PARP-1 to relevant target promoters to upregulate or downregulate gene transcription. In some cases, the enzymatic activity of PARP-1 is required (e.g., with c-fos and p53) [14–16], while in others it is not (e.g., RAR) [17]. (3) As a direct enhancer-binding factor, by binding to specific DNA sequences or structures in the regulatory regions of genes [18]. (4) As a regulator of insulators and insulator-binding factors. Chromatin insulators segregate the genome into expression domains by limiting the *cis*-effects of enhancers or silencers in a position-dependent manner [19]. For example, studies have demonstrated that PARP-1-dependent PARylation of CTCF, a ubiquitous DNA-binding protein that functions as an insulator, is in the preservation of insulator function [20].

In this study, we reveal a new molecular cooperation between Nrf2 and PARP-1 in the transcription of Nrf2 target genes. PARP-1 directly binds to both ARE and small Maf proteins directly, thereby enhancing Nrf2 binding to the ARE and upregulating Nrf2 target gene transcription. Transcriptional upregulation of nrf2 by PARP-1 neither involved a direct interaction between PARP-1 and Nrf2, nor the poly(ADP-ribose) polymerization activity of PARP-1. These findings reveal an additional mode of Nrf2 regulation.

Materials and Methods

Recombinant DNA molecules

Full length human PARP-1 was purchased from Open Biosystems (Thermo, IL), and was cloned into the pcDNA3.1 expression vector (Invitrogen, CA) using standard recombinant DNA technology. The PARP-1 DNA binding domain (PARP-1-DBD) construct was a generous gift from Dr. Yung Chang at Arizona State University. The construction of human NQO1-ARE TATA-Inr luciferase reporter plasmid and the mouse GSTA1-ARE TATA-Inr luciferase reporter plasmid were reported previously [21, 22]. Briefly, the 40 bp sequences containing ARE in the promoter regions of the *NQO1* or *GSTA1* genes were inserted into the pGL4.22 reporter plasmid using Mlu I and Bgl II restriction enzymes. The renilla luciferase plasmid pGL4.74 [hRluc/TK] was purchased from Promega (WI). The PARP-1-E988K construct was a generous gift from Dr. Scott H. Kaufmann at the University of Florida. PARP-1-ΔDBD was PCR amplified and inserted into the pcDNA3.1 expression vector (Invitrogen, CA) using EcoR I and Xho I restriction enzymes.

Cell culture and transfection

MDA-MB-231 and HEK293 cells were purchased from American Type Culture Collection (Manassas, VA). The PARP-$1^{+/+}$ and PARP-$1^{-/-}$ mouse embryonic fibroblast (MEF) cells were generous gifts from Dr. Myron K. Jacobson at the University of Arizona. Cells were maintained in either Eagle's minimal essential medium (MEM) or Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, CA) supplemented with 10% fetal bovine serum (FBS), 1% glutamine, and 0.1% gentamicin. All cells were incubated at 37°C in a humidified incubator containing 5% CO_2. Transfection of cDNA was performed using Lipofectamine Plus (Invitrogen, CA) according to the manufacturer's instructions. Short interfering RNA (siRNA) against PARP-1 and scrambled control siRNA were purchased from Qiagen. Transfection of 20 pmol siRNA was performed with HiPerfect (Qiagen, MD) according to the manufacturer's instructions.

Biotin-DNA pull-down

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (10 mM sodium phosphate pH 7.2, 150 mM NaCl, 1% sodium deoxycholate, 2 mM EDTA, 0.1% SDS, 1% NP-40) supplemented with 1mM phenylmethylsulfonyl fluoride (PMSF), 1mM DTT, and a protease inhibitor cocktail (Sigma, MO). Cell lysates were pre-cleared with protein A agarose beads and incubated with 2 μg biotinylated DNA probes that spanned the ARE-containing sequences in the promoter regions of *NQO1* and *HO-1*. The DNA-protein complexes were pulled down by streptavidin beads. After washing three times, the complexes were resolved on a SDS-PAGE gel and subjected to silver staining or immunoblot analysis. The sequences of the probes used are listed below: wild-type NQO1-ARE probe: 5'-AAATCGCAGTCACAGTGACTCAGCAGAATCTGAGCCTAGGG-3'; mutant NQO1-ARE probe: 5'-AAATCGCAGTCACAGACTCTCACGAGAATCTGAGCCTAGGG-3'.

Mass spectrometry analysis

Mass spectrometry analysis was performed by the Harvard Taplin mass spectrometry core facility. Briefly, biotin-DNA pulled down proteins were visualized by silver stain and recovered from the gel. Excised gel bands were cut into approximately 1 mm^3 pieces and sent for sequencing analysis.

Antibodies, immunoprecipitation and immunoblot analysis

Antibodies against Nrf2, Myc, GAPDH, β-actin, NQO1, HO-1, HA (Santa Cruz Biotechnology, CA), chitin-binding domain (CBD) (New England Biolabs, MA) and PARP-1 (Cell Signaling Technology, MA) were purchased from commercial sources. For detection of protein expression in total cell lysates, cells were washed with phosphate buffered salt (PBS) buffer and lysed with sample buffer containing 50 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 100 mM DTT, 0.1% bromophenol blue 48h post-transfection. Immunoprecipitation analyses were performed using cell lysates in RIPA buffer containing 1 mM DTT, 1 mM PMSF, and a protease inhibitor mixture (Sigma, MO). Cell lysates were incubated with 1 μg of antibodies and 20 μl of protein A agarose beads at 4°C overnight. Immunoprecipitated complexes were washed four times with RIPA buffer and eluted in sample buffer by boiling for 5 minutes. Samples were resolved by SDS-PAGE and transferred onto nitrocellulose membranes for immunoblot analyses.

Reporter gene assay

For the dual-luciferase reporter gene assay, indicated cells were transfected with expression plasmids for hNQO1-, mGSTA1-ARE, or κB-luciferase, along with the Renilla luciferase expression plasmid pGL4.74 (hRluc/TK) (Promega,WI) and c-Myc tagged full length PARP-1 (PARP-1-Myc-His) or DNA binding domain of PARP-1 (PARP-1-DBD) cDNA. For induction of Nrf2, cells were treated with known inducers of Nrf2, tert-butylhydroquinone (tBHQ) and sulforaphane (SF) (Sigma, MO). The DNA double-strand break inducer 1-Methyl-3-nitro-1-nitrosoguanidine (MNNG), was purchased from Sigma (MO) and the PARP-1 inhibitor, N-(6-Oxo-5,6-dihydrophenanthridin-2-yl)-(N,N-dimethylamino) acetamide hydrochloride (PJ-34), was a kind gift from Dr. Myron K. Jacobson at the University of Arizona. The other known PARP-1 inhibitor, 3-aminobenzamide (3-AB), was purchased from Sigma (MO). At 48h post-transfection, the cells were lysed with passive lysis buffer (Promega, WI) and both firefly and Renilla luciferase activities were measured with the dual-luciferase reporter assay system purchased from Promega (WI). Firefly luciferase activity was normalized to Renilla luciferase activity. The experiment was carried out in triplicate and repeated three times, and expressed as the mean ± the standard deviation (SD).

mRNA extraction and real-time qRT-PCR

Total mRNA was extracted using TRIzol (Invitrogen, CA) according to the manufacturer's instructions. Using equal amounts of mRNA and the Transcriptor First Strand cDNA synthesis kit (Roche, MD), cDNA was generated and used for real-time quantitative reverse transcription-PCR (qRT-PCR). The following TaqMan probes were obtained from the universal probe library (Roche, MD): human *Nrf2*, no. 70; *HO-1*, no. 25; *NQO1*, no. 87; GCLM, *no. 18*; *PARP-1*, no. 10 and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), no. 25. Both the forward and reverse primers for human *Nrf2*, *HO-1*, *NQO1* and *GAPDH* were synthesized by Integrated DNA Technologies and the sequences are as follow: *Nrf2*, ACACGGTCCACAGCTCATC (forward) and TGTCAATCAAATCCATGTCCTG (reverse); *HO-1*, AACTTTCAGAAGGGCCAGGT (forward) and CTGGGCTCTCCTTGTTGC (reverse); *NQO1*, ATGTATGACAAAGGACCCTTCC (forward) and TCCCTT GCAGAGAGTACATGG (reverse); *GCLM*,

GACAAAACACAGTTGGAACAGC (forward) and CAGTCAAATCTGGTGGCATC (reverse); *PARP-1*, ACAAGGCTTCCCTGTGCAT (forward) and CGGATGTTGGCTTCCTTTAC (reverse) and *GAPDH*, CTGACTTCAACAGCGACACC (forward) and TGCTGTAGCCAAATTCGTTGT (reverse). The real-time PCR was performed as followed: one cycle of pre-denaturation (94°C for 5 min), 40 cycles of amplification (94°C for 10 s and 60°C for 20 s), and a cooling program of 50°C for 30 s. Reactions for each sample were done in duplicate, and the experiment was repeated three times. The data are expressed as relative mRNA levels and were normalized to *GAPDH*.

Mobility shift assay

Mobility shift assays were conducted as previously described [21]. Purified recombinant Flag-PARP-1 was purchased from Active Motif (CA). One microliter (200 ng) Flag-PARP-1 protein was preincubated with poly(dI-dC) in binding buffer (50 mM HEPES, pH 7.5, 60 mM KCl, 2 mM $MgCl_2$, 0.004% NP-40, 5 mM EDTA, 10% glycerol, 100 μg/ml bovine serum albumin) for 10min at room temperature. ^{32}P-end-labeled DNA probes were added and further incubated for 20min before being loaded on a 4% native gel. The gel was dried and analyzed by autoradiography. For cold-probe competition, indicated concentrations of cold probe were used. The sequences of the probes were identical to those used in the biotin-DNA pull-down assay.

Chromatin immunoprecipitation (ChIP) assay

A ChIP assay was performed as previously reported [21, 23]. MDA-MB-231 cells (approximately 4×10^6) were cross-linked with formaldehyde, collected in PBS, resuspended in 200 μl SDS lysis buffer with PMSF and a protease inhibitor cocktail, and sonicated on ice. The lysates were then diluted in ChIP dilution buffer to 2 ml, precleared with protein A agarose, and then incubated with indicated antibodies or IgG control overnight. The immunoprecipitated complexes were collected with 50 μl protein A agarose, washed with low-salt buffer, high-salt buffer, LiCl buffer, and TE buffer. The complexes were eluted in 500 μl fresh elution buffer. The crosslinks were reversed by heating at 65°C for 5h after addition of 20 μl of 5 M NaCl. The samples were treated with RNase and proteinase K. DNA was recovered by phenol–chloroform extraction and ethanol precipitation. Relative amounts of DNA in the complex were quantified by the real-time PCR method using the LightCycler 480 DNA SYBR Green I kit (Roche). Primers used were as follows: human *NQO1* ARE forward, 5'-GCAGTCACAGTGACTCAGC-3'; human *NQO1* ARE reverse, 5'-TGTGCCCTGAGGTGCAA-3'; tubulin promoter forward, 5'-GTCGAGCCCTACAACTCTATC-3'; tubulin promoter reverse, 5'-CCGTCAAAGCGCAGAGAA-3'. PCR cycling was performed as follows: initial denaturation at 95°C for 5 min (1 cycle); 40 cycles of amplification at 95°C for 10 s, 60°C for 10 s, and 72°C for 20 s; with a single fluorescence acquisition. The amplification was followed by a melting curve program (65 to 95°C with a heating rate of 0.1°C per second and a continuous fluorescence measurement) and then a cooling program at 40°C for 30 s. The mean crossing-point values and standard deviations for *NQO1* and *tubulin* were determined for the different samples. The crossing point is defined as the point at which the fluorescence rises appreciably above the background fluorescence. A non-template control was run for each primer pair to assess the overall specificity and to ensure that primer dimers were not interfering with amplification detection. Amplification specificity was checked using melting curve and agarose gel electrophoresis. Melting-curve analysis showed a single sharp peak for all samples, and agarose gel electrophoresis showed a single band at the expected size. Data are presented as n-fold change. The real-time PCR assays were performed with triplicate samples.

Fluorescence polarization assay

Glutathione S-transferase (GST)-Nrf2 and His-MafG were expressed in Escherichia coli Rosetta (DE3) LysS cells and purified with glutathione Sepharose 4B matrix (GE Healthcare, Waukesha, WI) and Ni-NTA Agarose (Qiagen, Valencia, CA), separately. PARP-1 recombinant protein was purchased from Enzo Life Sciences (Farmingdale, NY).

Fluorescence polarization (FP) experiments were performed in black 96-well plates (Costar, Corning, NY). For the binding assay, 50 nM FAM-labeled human WT or MT NQO1-ARE (tracer) was mixed with Nrf2, MafG, PARP-1 or indicated combinations in 1× PBS buffer at a final volume of 100 μl. The plate was covered with adhesive aluminum foil and kept at room temperature for 2 h to reach equilibrium. The FP values were measured using a Synergy2 reader (BioTek, Winooski, VT) with excitation and emission wavelengths of 485/20 and 528/20 nm, respectively.

Statistical analysis

Data are presented as means ± SD. Differences were determined by two-tailed Student's t-test. A p value < 0.05 was considered significant.

RESULTS

PARP-1 binds the antioxidant response element (ARE)

In an attempt to identify proteins that associate with the antioxidant response element (ARE), MDA-MB-231 cells were left untreated or treated with 50 μM tBHQ for 16h. Whole-cell lysates were incubated with a biotinylated wild-type (WT) or mutant (MT) NQO1-ARE DNA (sequences shown in Figure 1A). ARE-bound proteins were pulled down by streptavidin beads, separated by SDS-PAGE, and visualized by silver stain. Gel pieces containing proteins that were present or increased in the WT NQO1-ARE, but not MT ARE, are indicated by the arrows in Figure 1B. The indicated bands were then isolated and subjected to mass spectrometry analysis using LC-MS/MS. Multiple proteins were detected (Figure 1C), including PARP-1 and small Maf proteins, a binding partner of Nrf2.

In order to confirm the results from the mass spectrometry analysis, a similar experiment was performed as the one used for identifying ARE-bound proteins. Biotinylated wild-type (WT) or mutant (MT) NQO1-ARE DNA was incubated with whole cell lysates from MDA-MB-231 cells that were either left untreated or treated with 50 μM tBHQ for 16h. ARE-bound proteins were pulled down by streptavidin beads and detected by immunoblot with anti-Nrf2 and anti-PARP-1 antibodies. Both PARP-1 and Nrf2 were pulled down in the presence of WT NQO1-ARE (Figure 1D). The MT NQO1-ARE only bound slightly to PARP-1 and there was no detectable binding with Nrf2 compared to the WT NQO1-ARE (Figure 1D, compare lane 1 to lane 2, lane 3 to lane 4), suggesting PARP-1 binds to the ARE in a sequence-specific manner. As expected, treatment with tBHQ increased the amount of Nrf2 both in total and in the pull-down assay (Figure 1D, compare lane 4 to lane 2). Collectively, these results demonstrate that binding of PARP-1 to the ARE is specific, and that this binding diminishes when the core sequence of the ARE is mutated.

PARP-1 enhances the transcriptional activity of Nrf2

To test the effect of PARP-1 binding to the ARE, the transcriptional activity of Nrf2 in the presence or absence of PARP-1 was measured in MDA-MB-231 cells. Cells were co-transfected with firefly luciferase reporter constructs driven by either WT or MT NQO1-ARE, together with PARP-1 alone or PARP-1 and Nrf2. At 48h post-transfection, luciferase reporter gene activities were measured. Overexpression of either PARP-1 or Nrf2 was sufficient to activate the transcription of NQO1-ARE-dependent luciferase that was further

enhanced by co-transfection of PARP-1 and Nrf2 (Figure 2A). MT NQO1-ARE was used as a negative control, and there were no changes in the MT NQO1-ARE luciferase activity (Figure 2A). In addition, both full length PARP-1 and the DNA binding domain of PARP-1 (PARP-1-DBD) enhanced the transcription of the luciferase reporter gene in the presence or absence of the Nrf2 activator sulforaphane (SF) in MDA-MB-231 cells (Figure 2B). The same effect was also observed in HEK293 cells (data not shown). This suggests that the upregulation of the transcriptional activity of Nrf2 by PARP-1 is not cell-type specific and that PARP-1-DBD is sufficient to enhance the transcription of Nrf2 target genes (Figure 2B).

Next, the specificity of the PARP-1-mediated enhancement on the ARE was examined. MDA-MB-231 cells were co-transfected with an expression vector for hNQO1-ARE-, mGSTA1-ARE-or a κB- dependent firefly luciferase reporter construct, along with a PARP-1-DBD expression plasmid. At 48h post-transfection, an increase in both the hNQO1-ARE and mGSTA1-ARE, but not the κB-dependent luciferase activity was observed. Moreover, transcription of the hNQO1-ARE- or mGSTA1-ARE- dependent luciferase gene was further enhanced in the presence of tBHQ or SF, whereas neither of them had any effect on the activity of the κB-dependent luciferase activity (Figure 2C), indicating that the effect of PARP-1 is enhancer-specific. Additionally, the hNQO1-ARE-dependent luciferase activity in PARP-$1^{+/+}$ and PARP-$1^{-/-}$ mouse embryonic fibroblast (MEF) cells was compared. Higher basal and induced NQO1-ARE-dependent luciferase activities were detected in PARP-$1^{+/+}$ cells compared to PARP-$1^{-/-}$ cells (Figure 2D). In a parallel set of experiments, a rescue assay was performed by transfecting full-length PARP-1 into PARP-$1^{-/-}$ cells. hNQO1-ARE-dependent luciferase activity in rescued cells was comparable to PARP-$1^{+/+}$ cells, although complete recovery was not achieved due to lack of 100% transfection efficiency (Figure 2D, comparing stripped bar to gray bar). In supporting of these data, we measured the expression of Nrf2 and its downstream genes *NQO1* and *GSTM1* in PARP-$1^{+/+}$ and PARP-$1^{-/-}$ MEF cells. PARP-$1^{-/-}$ MEF cells have both lower basal and induced expression levels of NQO1 and GSTM1, compared with PARP-$1^{+/+}$ MEF cells, whereas the Nrf2 levels in both cell lines were similar (Figure 2E). Taken together, these data suggest that PARP-1 enhances the transcriptional activity of Nrf2, and this effect is specific to the ARE containing promoters.

PARP-1 augments Nrf2-ARE binding and increases expression of Nrf2 target genes

Given that overexpression of PARP-1 increases the transcriptional activity of Nrf2, we speculated that PARP-1 plays a role in enhancing binding of Nrf2 with the ARE. Biotinylated NQO1-ARE DNA was incubated with whole cell lysates from MDA-MB-231 cells expressing PARP-1-Myc-His or PARP1-DBD. Cells were left untreated or were treated with 50 μM tBHQ for 16h. ARE-bound Nrf2 proteins were pulled down by streptavidin beads and detected by immunoblot with anti-Nrf2 antibodies. An increase in ARE binding was observed in cells expressing either form of PARP-1 compared to control, under both basal and induced conditions (Figure 3A, for quantification see bar graph below). Similar results were obtained in an ARE pull-down assay using cells ectopically overexpressing Nrf2 (data not shown), demonstrating that PARP-1 enhances the interaction between Nrf2 and the ARE.

Next, qRT-PCR was performed to confirm that enhanced Nrf2-ARE binding resulted in increased transcription of Nrf2 target genes. Overexpression of PARP-1 or PARP-1-DBD did not affect *Nrf2* mRNA levels (Figure 3B). In contrast, etopic expression of PARP-1 or PARP-1-DBD increased mRNA levels of *NQO1*, *HO-1*, *GCLM* and *AKR1C1* at both basal and induced conditions (Figure 3B). These results indicate that overexpression of PARP-1 or

PARP-1-DBD upregulates the transcriptional activity of Nrf2 under both basal and induced levels by enhancing Nrf2-ARE binding.

To confirm that the transcriptional upregulation of Nrf2 target genes leads to enhanced protein expression, immunoblot analyses were carried out in MDA-MB-231 cells transfected with an empty vector or with either an expression vector for PARP-1-Myc-His or PARP-1-DBD, and subsequently were either left untreated or treated with 50 μM tBHQ for 16h. Overexpression of either full-length PARP-1 or PARP-1-DBD had no effect on the protein level of Nrf2. However, the protein levels of NQO1, HO-1, GCLM and AKR1C1 were significantly increased in the presence of PARP-1 or PARP-DBD, at both basal and induced conditions (Figure 3C). The activation of Nrf2 target genes by PARP-1 or PARP-1-DBD was also confirmed in HEK293 cells (data not shown.

To determine whether depletion of endogenous PARP-1 affects Nrf2-dependent gene activation, MDA-MB-231 cells were co-transfected with either scrambled control siRNA or PARP-1 siRNA. Cells were left untreated or were treated with 50 μM tBHQ for 16h. qRT-PCR results suggest that knocking down PARP-1 decreases the transcription of Nrf2 target genes (*NQO1*, *HO-1*, *GCLM*, and *AKR1C1*) without significantly affecting the mRNA expression of Nrf2, under both basal and induced conditions (Figure 3D). Consistantly, immunoblot analysis confirmed that in the presence of PARP-1 siRNA, protein levels of NQO1, HO-1, GCLM and AKR1C1 decreased, whereas no change in the protein level of Nrf2 was observed (Figure 3E). Taken together, these data indicate that PARP-1 modulates transcriptional activation of Nrf2 target genes by enhancing Nrf2-ARE binding.

Nrf2 is not a substrate for poly(ADP-ribosyl)ation, and does not physically associate with PARP-1

PARP-1 is a known enzyme that is able to catalyze the polymerization of ADP-ribose units from donor NAD^+ molecules onto target proteins. Our finding that PARP-1-DBD is as effective as PARP-1 in upregulating the transcriptional activity of Nrf2 (Figure 3B and 3C) suggests that the enzymatic activity of PARP-1 is not required for its ability to modulate the Nrf2 transcriptional activity. In support of this notion, transfected MDA-MB-231 cells were treated with increasing doses of PJ-34 or 3-AB, both of which are well-documented PARP-1 inhibitors. Reporter gene analysis indicates neither PJ-34 nor 3-AB affected NQO1-ARE-dependent luciferase activity under basal or induced conditions (Figure 4A), suggesting that PARP-1-mediated upregulation of Nrf2 transcriptional activity is independent of the enzymatic activity of PARP-1. To further confirm this, PARP-$1^{-/-}$ cells were transfected with an enzymatic inactive PARP-1 mutant – PARP-1-E988K [24, 25], to test if it could complement PARP-1 function in PARP-$1^{-/-}$ cells. Similar to what was observed for PARP-1 in rescuing the transcriptional activity of Nrf2 (Figure 2D), PARP-1-E988K reverted the NQO1-ARE-dependent luciferase activity to the comparable level as in PARP-$1^{+/+}$ cells, These results suggest that PARP-1 enzyme activity is dispensable for the transcriptional activity of Nrf2 (Figure 4B).

An immunoprecipitation assay was carried out to test if Nrf2 is (ADP-ribosyl)ated by PARP-1. PARP-$1^{-/-}$ cells were transfected with HA-Nrf2 alone or co-transfected with HA-Nrf2 and myc/his-PARP-1. As a positive control, a parallel set of cells were transfected with GFP-p53 or co-transfected with GFP-p53 and myc/his-PARP-1. At 36h post-transfection, cell lysates from PARP-$1^{-/-}$ MEFs transfected with HA-Nrf2 or GFP-p53 were immunoprecipitated with an anti-HA or anti-GFP antibody, and immunoblotted with anti-GFP, anti-myc and anti-poly(ADPribose) antibodies. A fair amount of (ADP-ribosyl)ated GFP-p53 was detected, whereas HA-Nrf2 was not poly(ADP-ribosyl)ated (Figure 4C). These data suggest that Nrf2 is not a substrate for PARP-1 mediated poly(ADP-

ribosyl)ation, and that PARP-1 regulates the transcriptional activity of Nrf2 in a poly(ADP-ribosyl)ation-independent manner.

Next, the interaction between PARP-1 and Nrf2 was examined. HEK293 cells were transfected with an expression vector for PARP-his or Keap1-CBD (positive control) with or without an expression vector for HA-Nrf2. Keap1-CBD, but not full length PARP-1, was immunoprecipitated by HA-Nrf2 (Figure 4D), suggesting that there is no association between Nrf2 and PARP-1.

PARP-1 directly interacts with MafG

Next, whether PARP-1 interacts with MafG and thus enhances Nrf2-ARE binding was tested. PARP-$1^{-/-}$ cells transfected with Myc-MafG, or co-transfected with Myc-MafG and PARP-1, were subjected to immunoprecipitation analysis with a PARP-1 antibody. MafG was immunoprecipitated by PARP-1 (Figure 5A, left panel). The association between PARP-1 and MafG was also detected in a reciprocal immunoprecipitation analysis using an anti-myc antibody to immunoprecipitate PARP-1 (Figure 5A, right panel). To further confirm the interaction between endogenous Maf and PARP-1 proteins, immunoprecipitation analysis was performed with anti-MafG antibodies or normal rabbit IgG (negative control). Both PARP-1 and Nrf2 were detected in the MafG immunoprecipitated complex (Figure 5B), suggesting that PARP-1 associates with MafG (Figure 4D). Next, an *in vitro* pulldown assay was carried out to examine whether there is a direct interaction between MafG and PARP-1. Purified GST-Nrf2 or His-MafG were incubated with or without Flag-PARP-1. PARP-1-bound proteins were pulled down by Flag-M2 beads and subject to immunoblot with anti-GST and anti-His antibodies. MafG but not Nrf2 was pulled down by Flag-PARP-1 (Figure 5C), suggesting a direct physical interaction between MafG and PARP-1.

To further confirm that PARP-1 enhances Nrf2 binding to the ARE through direct interaction with MafG, an *in vitro* binding assay using biotinylated NQO1-ARE DNA was performed. Biotinylated wild-type or mutant NQO1-ARE DNA was incubated at 4 °C with purified GST-Nrf2, PARP-1, His-MafG or the indicated combinations for 4h. ARE-bound proteins were pulled down by streptavidin beads and detected by immunoblot with anti-GST (for Nrf2), anti-PARP-1 or anti-His (for MafG) antibodies. As expected, Nrf2 bound to WT NQO1-ARE only in the presence of MafG (Figure 5D, compare lane 1 with lane 2). Enhanced Nrf2-ARE binding was observed in the presence of PARP-1 (Figure 5D, compare lane 2 with lane 3). No binding of Nrf2 to the MT ARE was detected (Figure 5D, lane 7 to lane 12). Not surprisingly, MafG alone was sufficient to bind to WT but not MT NQO1-ARE (Figure 5D, lane 4 and lane 10), since the Maf homodimer is capable of binding to the ARE. Interestingly, WT NQO1-ARE has greater binding affinity to PARP-1 than MT NQO1-ARE, when purified PARP-1 was used for the binding assay (Figure 5D, compare lane 6 with lane 12). The small amount of PARP-1 binding to MT NQO1-ARE is likely a result of PARP-1 binding to nicked DNA – the end of oligos, in our case. Nrf2 was not pulled down by WT or MT NQO1-ARE in the presence of PARP-1 (Figure 5D, lane 5 and lane 11), which further supports our hypothesis that Nrf2 does not interact with PARP-1.

The fact that purified PARP-1 binds the WT ARE more than the MT ARE led us to test the possible direct binding of PARP-1 with the ARE, using a mobility shift assay. The NQO1-ARE probe was radiolabeled and incubated with PARP-1. Flag-PARP-1 protein formed a complex with WT NQO1-ARE (Figure 5E, lane 3), which could be competed by increasing amounts of non-radiolabeled WT but not MT NQO1-ARE probe (Figure 5E, compare lane 3–7), suggesting that PARP-1 specifically binds to the ARE. A supershift assay using an anti-PARP-1 antibody further supports the DNA binding specificity by PARP-1 (Figure 5E,

lane 8 and 9). Taken together, these data suggest that PARP-1 binds directly to both MafG and the ARE and enhances the binding of Nrf2-Maf binding to the ARE.

To confirm the EMSA results, a ChIP assay was performed to verify that PARP-1 associates with the ARE *in vivo*. Quantification of the immunoprecipitated DNA fragments was performed using a real-time PCR method. MDA-MB-231 cells were left untreated or treated with tBHQ for 16h. Chromatin DNA bound by PARP-1 or Nrf2 was immunoprecipitated with anti-PARP-1 or anti-Nrf2 antibodies. Incubation with IgG was included as a negative control. The precipitated DNAs were recovered and used as templates for amplification of the ARE core-containing NQO1 promoter region or the promoter region of tubulin using sequence-specific primer pairs. As shown in Figure 5F, the NQO1-ARE was immunoprecipitated with the anti-Nrf2 antibody in the untreated cells, indicating the constitutive binding with Nrf2. tBHQ increased the association of the ARE with Nrf2 by approximately two fold. There was no amplification of the anti-tubulin promoter in any anti-Nrf2 immunoprecipitated samples, indicating that endogenous Nrf2 binds specifically to the ARE sequence under both basal and induced conditions. Both NQO1-ARE and tubulin promoters were immunoprecipitated with the anti-PARP-1 antibody. However, the enrichment of NQO1-ARE was about two-fold more than the tubulin promoter, which is consistent with our observations in Figure 1D and Figure 5D that PARP-1 preferentially binds to WT NQO1-ARE. Noticeably, tBHQ treatment mildly decreased the enrichment of PARP-1 at both NQO1-ARE and tubulin promoter, consistent with data in Figure 1D, suggesting that tBHQ may affect PARP-1 DNA binding in general.

To further confirm these results, a fluorescence-based polarization (FP) assay was utilized to test the binding of these proteins with the WT and MT NQO1-ARE. Binding of a protein to a fluorescence-labeled DNA tracer enhances the polarization value (mP). Since it is known that small Maf proteins can form homodimers that bind with the ARE, we titrated the concentration of MafG to minimize the homodimer formation as illustrated by the slight increase of the mP value of MafG alone, compared to tracer only (Figure 5G). As expected, Nrf2 alone had no effect on mP. In the presence of both Maf and Nrf2 an increase in mP was observed, an effect that was further enhanced by the addition of PARP-1 (Figure 5G), suggesting that the protein complex containing PARP-1, MafG and Nrf2 binds to the WT NQO1-ARE better than the Maf-Nrf2 dimer. Noticeably, PARP-1 alone increased the mP value of both WT and MT NQO1-ARE, probably because PARP-1 can bind to DNA ends. However, PARP-1 binds preferably to WT ARE, suggesting possible sequence preference. This preference was maximized in the PARP-1/MafG/Nrf2 combination group, suggesting that PARP-1 enhances sequence specific binding of the MafG-Nrf2 heterodimer with NQO1-ARE.

DISCUSSION

Although formation of the Nrf2-Maf heterodimer is the “grand switch” for ARE binding and activation of target gene transcription, several other proteins have been shown to interact with Nrf2 and modulate Nrf2-ARE signaling. Previously, co-activators such as the p300/CBP complex, was identified as a modulator of the Nrf2 signaling pathway [26]. In response to arsenite-induced oxidative stress, p300/CBP directly binds to Nrf2 and acetylates it at multiple lysine residues, and thereby augments promoter-specific DNA binding of Nrf2. In another case, nuclear coregulator RAC3, a member of the p160 cofactor family, was shown to bind to the Nrf2 protein directly in the nucleus and enhance transcription of HO-1 [27, 28]. In addition, c-jun was identified as a component of the ARE–nuclear protein complex. C-jun forms a heterodimer with Nrf2 and is responsible for the induction of NQO1 gene expression [29]. All these findings suggest that Nrf2 can coordinate with other transcription

factors/co-factors in binding to the AREs, and thus regulate Nrf2 transcriptional activity. However, how these co-factors are recruited.

In this study, we demonstrate that PARP-1 functions as a co-activator to enhance the transcription activity of Nrf2. PARP-1 is a multifunctional nuclear protein that is involved in the detection and signaling of DNA strand breaks introduced by oxidative stress, ionizing radiation, and cytotoxic agents. PARP-1 is also implicated in other cellular processes, such as DNA repair and maintenance of genomic integrity [30], regulation of protein expression at the transcriptional level [12], and modulation of cell death via necrosis and apoptosis [31, 32].

PARP-1 has been shown to regulate transcription of genes through multiple mechanisms, depending on the presence of specific binding partners, the proliferative status of the cell, the concentration of NAD^+ and the presence of DNA strand breaks [33]. In some cases, PARP-1 enzymatic activity is required for its regulatory activity. For example, in the presence of NAD^+, PARP-1-dependent silencing of transcription involves poly(ADP-ribosyl)ation of specific transcription factors, such as p53 and c-fos [34, 35]. However, in other occasions, its enzymatic activity is not required. For example, the repression of the heat shock response by HSF-1 requires physical interaction with PARP-1 but not its catalytic activity [36]. However, the detailed mechanism by which PARP-1 modulates transcriptional activity of different transcription factors remains poorly understood.

Two major modes of how PARP-1 regulates transcriptional activity have been proposed: (1) a histone-modifying enzymatic activity that can regulate chromatin structure and (2) an enhancer/promoter binding co-regulator activity that can coordinate with other transcription co-factors. In the first model, PARP-1 modulates chromatin by directly (ADP-ribosyl)ating core histones and chromatin associated proteins, thereby promoting the dissociation of nucleosomes and the decondensation of chromatin [11]. A specific example supporting this model is puff formation in Drosophila polytene chromosomes, which presents PARP-1-dependent accumulation of PAR at decondensed, transcriptionally active loci [37, 38]. PARP-1 can also poly(ADP-ribosyl)ate DEK, another repressive chromatin-associated protein, and evict it from chromatin, consequently allowing access of the transcription machinery to the chromatin [39]. In the “coregulator” mode, PARP-1 may be recruited to the specific promoters by interactions with DNA binding factors [40], or may contact the enhancer/promoter directly by recognizing the DNA structure or sequence [41, 42]. It can either stimulate the activity of transcriptional factors, or repress transcription in a promoter and cell-type specific manner. For example, PARP-1 was shown to bind to B-MYB and promote its transcriptional activity [43], whereas direct binding to retinoid X receptors (RXR) by PARP-1 repressed ligand-dependent transcriptional activities mediated by heterodimers of RXR and thyroid hormone receptor (TR) [44]. Complicating the model even further is to determine whether the enzymatic activity of PARP-1 is required for its regulatory functions. In some cases, PARP-1 mediated poly(ADP-ribosyl)ation is indispensable for its co-activator/co-repressor functions [44, 45], while in other cases, the enzymatic activity of PARP-1 is not required [43]. All these findings suggest that the action of PARP-1 as a transcriptional co-regulator is complex.

Our current study reveals a potential mechanism that may fall into the second mode of PARP-1 transcriptional regulation described above. Based on the biotin-ARE pulldown and mass spectrometry analysis, PARP-1 was shown to be associated with the ARE within the promoter of NQO1 (Figure 1), augment binding between Nrf2 and the ARE, and increase the transcription of Nrf2 target genes (Figure 2 and 3). No physical interaction was found between Nrf2 and PARP-1 (Figure 4), nor was the catalytic activity of PARP-1 required for transcriptional activation of Nrf2 (Figure 4). The direct interaction between PARP-1 and

MafG was confirmed both *in vivo* and *in vitro* (Figure 5A to 5C), and this interaction enhanced the binding between Nrf2 and the ARE (Figure 5D and 5G). In addition, PARP-1 was able to bind with the ARE specifically (Figure 5E and 5F). Therefore, we conclude that PARP-1 does not directly interact with Nrf2, but is able to enhance Nrf2 binding to the ARE by directly interacting with both MafG and the ARE, thereby increasing Nrf2 target gene expression.

In summary, this study indicates that PARP-1 may serve as a transcriptional co-activator of MafG-Nrf2 by directly binding to the ARE and MafG, thereby enhancing binding of the heterodimer with the ARE (Figure 6). Our study reveals a novel function of PARP-1 in upregulating Nrf2 transcriptional activity and Nrf2 target gene expression. However, the physical or pathological relevance of this PARP-1-mediated transcriptional modulation remains to be investigated. Whether PARP-1 recruits other transcription co-factors or mediators to facilitate transcription is still unclear. This type of complexity reflected best in the regulation of the expression of HO-1. Although our data suggested that PARP-1 upregulates both basal and inducible expression of HO-1, Katoh et al. reported that PARP-1 serves as a transcription corepressor of MafK in a mouse plasmacytoma cell line X63/0 [46], in which PARP-1 interacts with a protein complex composed of MATIIa, Polycomb group (PcG), NuRD, Swi/Snf. The seemly contradictory results indicate that the role of PARP-1 is complicated and conclusions need to be drawn with precaution based on the physiological status of the cell. In addition, there is also evidence suggesting that Bach1 represses HO-1 expression [47, 48], which makes the effects of PARP-1 even more complicated. No previous study showed that the repressive effect of Bach1 on HO-1 transcription is due to antagonization by PARP-1, and therefore it remained to be explored that how PARP-1 cross-talks with other signal transduction molecules in a more profound context.

Acknowledgments

We thank Dr. Myron K. Jacobson, Dr. Yung Chang and Dr. Scott H Kaufmann for their kind help and reagents.

REFERENCES

[1]. Wild AC, Moinova HR, Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. J Biol Chem. 1999; 274:33627–33636. [PubMed: 10559251]

[2]. Thimmulappa RK, Mai KH, Srisuma S, Kensler TW, Yamamoto M, Biswal S. Identification of Nrf2-regulated genes induced by the chemopreventive agent sulforaphane by oligonucleotide microarray. Cancer Res. 2002; 62:5196–5203. [PubMed: 12234984]

[3]. Tanito M, Agbaga MP, Anderson RE. Upregulation of thioredoxin system via Nrf2-antioxidant responsive element pathway in adaptive-retinal neuroprotection in vivo and in vitro. Free Radic Biol Med. 2007; 42:1838–1850. [PubMed: 17512463]

[4]. Hayes JD, Chanas SA, Henderson CJ, McMahon M, Sun C, Moffat GJ, Wolf CR, Yamamoto M. The Nrf2 transcription factor contributes both to the basal expression of glutathione S-transferases in mouse liver and to their induction by the chemopreventive synthetic antioxidants, butylated hydroxyanisole and ethoxyquin. Biochem Soc Trans. 2000; 28:33–41. [PubMed: 10816095]

[5]. Enomoto A, Itoh K, Nagayoshi E, Haruta J, Kimura T, O'Connor T, Harada T, Yamamoto M. High sensitivity of Nrf2 knockout mice to acetaminophen hepatotoxicity associated with decreased expression of ARE-regulated drug metabolizing enzymes and antioxidant genes. Toxicol Sci. 2001; 59:169–177. [PubMed: 11134556]

[6]. Moinova HR, Mulcahy RT. Up-regulation of the human gamma-glutamylcysteine synthetase regulatory subunit gene involves binding of Nrf-2 to an electrophile responsive element. Biochem Biophys Res Commun. 1999; 261:661–668. [PubMed: 10441483]

[7]. Xu C, Li CY, Kong AN. Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch Pharm Res. 2005; 28:249–268. [PubMed: 15832810]

[8]. Ohta K, Ohigashi M, Naganawa A, Ikeda H, Sakai M, Nishikawa J, Imagawa M, Osada S, Nishihara T. Histone acetyltransferase MOZ acts as a co-activator of Nrf2-MafK and induces tumour marker gene expression during hepatocarcinogenesis. Biochem J. 2007; 402:559–566. [PubMed: 17083329]

[9]. Shenvi SV, Smith E, Hagen TM. Identification of age-specific Nrf2 binding to a novel antioxidant response element locus in the Gclc promoter: a compensatory means for the loss of glutathione synthetic capacity in the aging rat liver? Aging Cell. 2012; 11:297–304. [PubMed: 22212472]

[10]. Krishnakumar R, Kraus WL. The PARP side of the nucleus: molecular actions, physiological outcomes, and clinical targets. Mol Cell. 2010; 39:8–24. [PubMed: 20603072]

[11]. Kraus WL, Lis JT. PARP goes transcription. Cell. 2003; 113:677–683. [PubMed: 12809599]

[12]. Kim MY, Mauro S, Gevry N, Lis JT, Kraus WL. NAD+-dependent modulation of chromatin structure and transcription by nucleosome binding properties of PARP-1. Cell. 2004; 119:803–814. [PubMed: 15607977]

[13]. Huletsky A, de Murcia G, Muller S, Hengartner M, Menard L, Lamarre D, Poirier GG. The effect of poly(ADP-ribosyl)ation on native and H1-depleted chromatin. A role of poly(ADP-ribosyl)ation on core nucleosome structure. J Biol Chem. 1989; 264:8878–8886. [PubMed: 2498319]

[14]. Amstad PA, Krupitza G, Cerutti PA. Mechanism of c-fos induction by active oxygen. Cancer Res. 1992; 52:3952–3960. [PubMed: 1617671]

[15]. Huang D, Wang Y, Yang C, Liao Y, Huang K. Angiotensin II promotes poly(ADP-ribosyl)ation of c-Jun/c-Fos in cardiac fibroblasts. J Mol Cell Cardiol. 2009; 46:25–32. [PubMed: 19027749]

[16]. Wang X, Ohnishi K, Takahashi A, Ohnishi T. Poly(ADP-ribosyl)ation is required for p53-dependent signal transduction induced by radiation. Oncogene. 1998; 17:2819–2825. [PubMed: 9879988]

[17]. Pavri R, Lewis B, Kim TK, Dilworth FJ, Erdjument-Bromage H, Tempst P, de Murcia G, Evans R, Chambon P, Reinberg D. PARP-1 determines specificity in a retinoid signaling pathway via direct modulation of mediator. Mol Cell. 2005; 18:83–96. [PubMed: 15808511]

[18]. Amiri KI, Ha HC, Smulson ME, Richmond A. Differential regulation of CXC ligand 1 transcription in melanoma cell lines by poly(ADP-ribose) polymerase-1. Oncogene. 2006; 25:7714–7722. [PubMed: 16799643]

[19]. Wallace JA, Felsenfeld G. We gather together: insulators and genome organization. Curr Opin Genet Dev. 2007; 17:400–407. [PubMed: 17913488]

[20]. Yu W, Ginjala V, Pant V, Chernukhin I, Whitehead J, Docquier F, Farrar D, Tavoosidana G, Mukhopadhyay R, Kanduri C, Oshimura M, Feinberg AP, Lobanenkov V, Klenova E, Ohlsson R. Poly(ADP-ribosyl)ation regulates CTCF-dependent chromatin insulation. Nat Genet. 2004; 36:1105–1110. [PubMed: 15361875]

[21]. Sun Z, Zhang S, Chan JY, Zhang DD. Keap1 controls postinduction repression of the Nrf2-mediated antioxidant response by escorting nuclear export of Nrf2. Mol Cell Biol. 2007; 27:6334–6349. [PubMed: 17636022]

[22]. Wang XJ, Sun Z, Chen W, Eblin KE, Gandolfi JA, Zhang DD. Nrf2 protects human bladder urothelial cells from arsenite and monomethylarsonous acid toxicity. Toxicol Appl Pharmacol. 2007; 225:206–213. [PubMed: 17765279]

[23]. Zhao F, Wu T, Lau A, Jiang T, Huang Z, Wang XJ, Chen W, Wong PK, Zhang DD. Nrf2 promotes neuronal cell differentiation. Free Radic Biol Med. 2009; 47:867–879. [PubMed: 19573594]

[24]. Buelow B, Uzunparmak B, Paddock M, Scharenberg AM. Structure/function analysis of PARP-1 in oxidative and nitrosative stress-induced monomeric ADPR formation. PLoS One. 2009; 4:e6339. [PubMed: 19641624]

[25]. Hassa PO, Covic M, Hasan S, Imhof R, Hottiger MO. The enzymatic and DNA binding activity of PARP-1 are not required for NF-kappa B coactivator function. J Biol Chem. 2001; 276:45588–45597. [PubMed: 11590148]

[26]. Sun Z, Chin YE, Zhang DD. Acetylation of Nrf2 by p300/CBP augments promoter-specific DNA binding of Nrf2 during the antioxidant response. Mol Cell Biol. 2009; 29:2658–2672. [PubMed: 19273602]

[27]. Lin W, Shen G, Yuan X, Jain MR, Yu S, Zhang A, Chen JD, Kong AN. Regulation of Nrf2 transactivation domain activity by p160 RAC3/SRC3 and other nuclear co-regulators. J Biochem Mol Biol. 2006; 39:304–310. [PubMed: 16756760]

[28]. Kim JH, Yu S, Chen JD, Kong AN. The nuclear cofactor RAC3/AIB1/SRC-3 enhances Nrf2 signaling by interacting with transactivation domains. Oncogene. 2012

[29]. Jaiswal AK. Regulation of genes encoding NAD(P)H:quinone oxidoreductases. Free Radic Biol Med. 2000; 29:254–262. [PubMed: 11035254]

[30]. Shall S, de Murcia G. Poly(ADP-ribose) polymerase-1: what have we learned from the deficient mouse model? Mutat Res. 2000; 460:1–15. [PubMed: 10856830]

[31]. Schreiber V, Dantzer F, Ame JC, de Murcia G. Poly(ADP-ribose): novel functions for an old molecule. Nat Rev Mol Cell Biol. 2006; 7:517–528. [PubMed: 16829982]

[32]. Bouchard VJ, Rouleau M, Poirier GG. PARP-1, a determinant of cell survival in response to DNA damage. Exp Hematol. 2003; 31:446–454. [PubMed: 12829019]

[33]. Garcia S, Bodano A, Pablos JL, Gomez-Reino JJ, Conde C. Poly(ADP-ribose) polymerase inhibition reduces tumor necrosis factor-induced inflammatory response in rheumatoid synovial fibroblasts. Ann Rheum Dis. 2008; 67:631–637. [PubMed: 17890271]

[34]. Wesierska-Gadek J, Schmid G. Poly(ADP-ribose) polymerase-1 regulates the stability of the wild-type p53 protein. Cell Mol Biol Lett. 2001; 6:117–140. [PubMed: 11544635]

[35]. Cohen-Armon M. PARP-1 activation in the ERK signaling pathway. Trends Pharmacol Sci. 2007; 28:556–560. [PubMed: 17950909]

[36]. Fossati S, Formentini L, Wang ZQ, Moroni F, Chiarugi A. Poly(ADP-ribosyl)ation regulates heat shock factor-1 activity and the heat shock response in murine fibroblasts. Biochem Cell Biol. 2006; 84:703–712. [PubMed: 17167533]

[37]. Kolesnikova TD, Semeshin VF, Andreyeva EN, Zykov IA, Kokoza EB, Kalashnikova DA, Belyaeva ES, Zhimulev IF. Induced decondensation of heterochromatin in Drosophila melanogaster polytene chromosomes under condition of ectopic expression of the Supressor of underreplication gene. Fly (Austin). 2011; 5:181–190. [PubMed: 21747232]

[38]. Tulin A, Spradling A. Chromatin loosening by poly(ADP)-ribose polymerase (PARP) at Drosophila puff loci. Science. 2003; 299:560–562. [PubMed: 12543974]

[39]. Gamble MJ, Fisher RP. SET and PARP1 remove DEK from chromatin to permit access by the transcription machinery. Nat Struct Mol Biol. 2007; 14:548–555. [PubMed: 17529993]

[40]. Lee D, Kim JW, Kim K, Joe CO, Schreiber V, Menissier-De Murcia J, Choe J. Functional interaction between human papillomavirus type 18 E2 and poly(ADP-ribose) polymerase 1. Oncogene. 2002; 21:5877–5885. [PubMed: 12185587]

[41]. Akiyama T, Takasawa S, Nata K, Kobayashi S, Abe M, Shervani NJ, Ikeda T, Nakagawa K, Unno M, Matsuno S, Okamoto H. Activation of Reg gene, a gene for insulin-producing beta -cell regeneration: poly(ADP-ribose) polymerase binds Reg promoter and regulates the transcription by autopoly(ADP-ribosyl)ation. Proc Natl Acad Sci U S A. 2001; 98:48–53. [PubMed: 11134536]

[42]. Plaza S, Aumercier M, Bailly M, Dozier C, Saule S. Involvement of poly (ADP-ribose)-polymerase in the Pax-6 gene regulation in neuroretina. Oncogene. 1999; 18:1041–1051. [PubMed: 10023680]

[43]. Cervellera MN, Sala A. Poly(ADP-ribose) polymerase is a B-MYB coactivator. J Biol Chem. 2000; 275:10692–10696. [PubMed: 10744766]

[44]. Miyamoto T, Kakizawa T, Hashizume K. Inhibition of nuclear receptor signalling by poly(ADP-ribose) polymerase. Mol Cell Biol. 1999; 19:2644–2649. [PubMed: 10082530]

[45]. Butler AJ, Ordahl CP. Poly(ADP-ribose) polymerase binds with transcription enhancer factor 1 to MCAT1 elements to regulate muscle-specific transcription. Mol Cell Biol. 1999; 19:296–306. [PubMed: 9858553]

[46]. Katoh Y, Ikura T, Hoshikawa Y, Tashiro S, Ito T, Ohta M, Kera Y, Noda T, Igarashi K. Methionine adenosyltransferase II serves as a transcriptional corepressor of Maf oncoprotein. Mol Cell. 2011; 41:554–566. [PubMed: 21362551]

[47]. Kassovska-Bratinova S, Yang G, Igarashi K, Dennery PA. Bach1 modulates heme oxygenase-1 expression in the neonatal mouse lung. Pediatr Res. 2009; 65:145–149. [PubMed: 18948842]

[48]. Igarashi K, Sun J. The heme-Bach1 pathway in the regulation of oxidative stress response and erythroid differentiation. Antioxid Redox Signal. 2006; 8:107–118. [PubMed: 16487043]

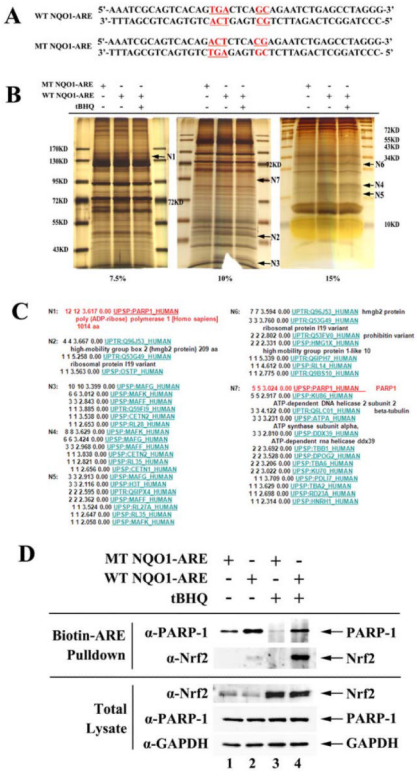

FIGURE 1.

PARP-1 binds the NQO1-ARE. (A) Sequences of wild-type (ARE) and mutant NQO1-ARE (mARE) used in this study. Nucleotides labeled in red are mutated in mARE. (B) Silver stain of proteins pulled down by the biotinylated ARE or mARE pull-downs/streptavidin beads. MDA-MB-231 cells were left untreated or were treated with 50 μM tBHQ for 16h. Cell lysates were incubated with the indicated biotinyaled-NQO1-ARE. The proteins were pulled down using streptavidin beads and separated on SDS-PAGE gels and subjected to silver staining. Arrows indicate proteins that were only present or increased in the wild-type NQO1-ARE samples, but not the mutant ARE. (C) Identification of ARE-bound proteins by LC-MS/MS mass spectrometry analysis. (D) Association between PARP-1 and the ARE. Biotinylated ARE and mARE were incubated with whole cell lysates from MDA-MB-231 cells either left untreated or treated with 50 μM tBHQ for 16h. ARE-bound proteins were pulled down by streptavidin beads and detected by immunoblot analysis with anti-Nrf2 and anti-PARP-1 antibodies.

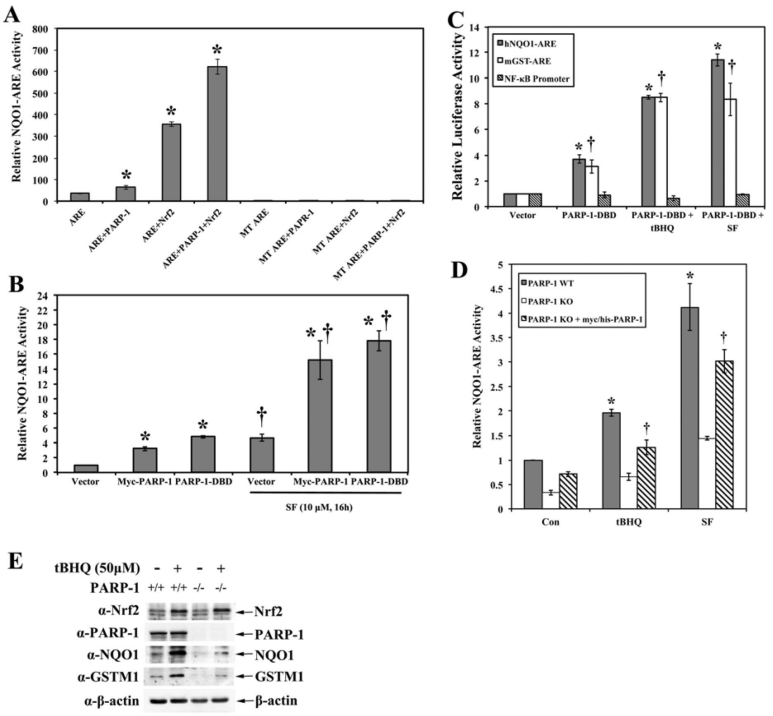

FIGURE 2.

PARP-1 upregualtes the transcriptional activity of Nrf2. (A) PARP-1 promotes the NQO1-ARE-dependent luciferase activity. MDA-MB-231 cells were co-transfected with expression vectors for either wild-type or mutant NQO1-ARE-dependent firefly luciferase and TK-Renilla luciferase, alone with Nrf2, PARP-1, or both. Luciferase reporter gene activities were analyzed at 36h post-transfection. Relative luciferase activities and standard deviations were calculated from three independent experiments. (B) Both full-length PARP-1 and PARP-DBD enhanced NQO1-ARE-dependent luciferase activity. Reporter gene assays were performed in MDA-MB-231 cells as described in Figure 1. (C) The PARP-1-mediated transcriptional upregulation is promoter specific. MDA-MB-231 cells were co-transfected with hNQO1-ARE-, mGSTA1-ARE- or κB- dependent firefly luciferase reporter genes, along with an expression plasmid for PARP-1-DBD. At 24h post-transfection, cells were treated with either tBHQ or SF for an additional 16h prior to measurement of luciferase activities. (D) Higher NQO1-ARE promoter activity was detected in PARP-$1^{+/+}$ cells than PARP-$1^{-/-}$ cells. Overexpression of full-length PARP-1 could rescue transcription of Nrf2 target genes. PARP-$1^{+/+}$ and PARP-$1^{-/-}$ MEF cells were transfected with expression vectors for NQO1-ARE-dependent firefly luciferase and TK-Renilla luciferase. Cells were then treated with tBHQ or SF for 16h before measurement of luciferase activities. For rescue assay, PARP-$1^{-/-}$ MEF cells were co-transfected with myc/his-PARP-1, firefly luciferase and TK-Renilla luciferase. (E) Higher NQO1 and GSTM1 expression were detected in PARP-$1^{+/+}$ cells than PARP-$1^{-/-}$ cells. PARP-$1^{+/+}$ and PARP-$1^{-/-}$ MEF cells were left untreated or treated 50 μM tBHQ for 16h, before harvest for immunoblot assay with indicated antibodies.

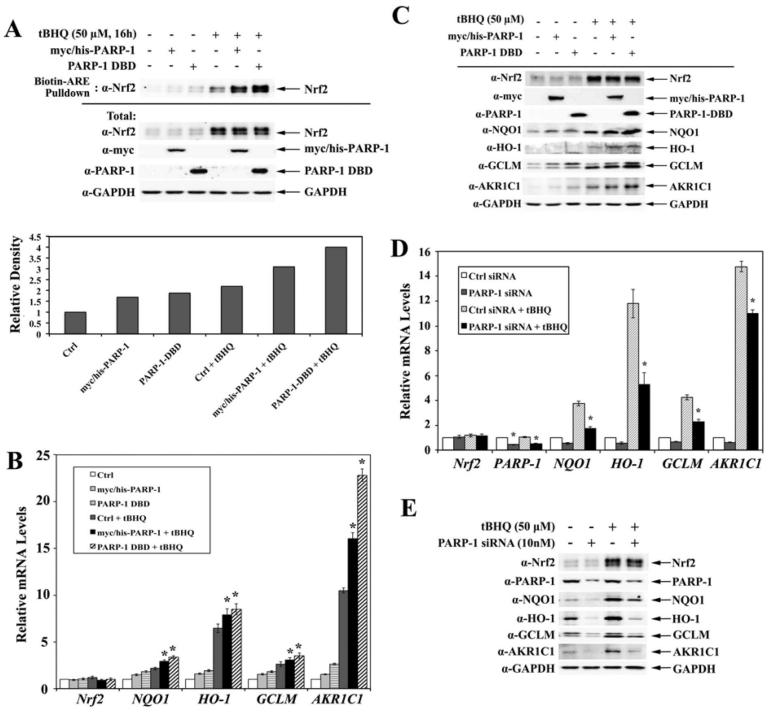

FIGURE 3.

PARP-1 augments Nrf2 binding to the ARE and upregulates expression of Nrf2 target genes. (A) PARP-1 enhances the binding of endogenous Nrf2 to the NQO1-ARE. Biotinylated NQO1-ARE DNA was incubated with whole cell lysates from MDA-MB-231 cells expressing empty vector, his/myc-PARP-1 or PARP1-DBD. Cells were untreated or treated with 50 μM tBHQ for 16h. ARE-bound Nrf2 proteins were pulled down by streptavidin beads and detected by immunoblot analysis with anti-Nrf2 antibodies. Densitometry of biotin pull-down was showed in the lower panel. (B) PARP-1 increases the transcription of Nrf2 target genes. MDA-MB-231 cells were transfected with vector, myc/his-PARP-1 or PARP-1-DBD as described previously. At 24h post-transfection, cells were treated with 50μM tBHQ for 16 h and mRNA was extracted for qRT-PCR analysis. Error bars indicate standard deviations from three independent experiments. (C) PARP-1 upregualtes Nrf2 target gene expression in MDA-MB-231 cells. Cells were transfected with empty vector, myc/his-PARP-1 or PARP-1-DBD, and treated with 50 μM tBHQ. Cell lysates were then subjected to immunoblot analysis with indicated antibodies. (D) Knockdown of PARP-1 diminishes Nrf2 target gene transcription. MDA-MB-231 cells were transfected with scrambled or PARP-1 siRNA, and then either left untreated or treatedwith 50 μM tBHQ for 16h, before havest for qPCR analysis. Error bars indicate standard deviations from three independent experiments. (E) Knockdown of PARP-1 decreases Nrf2 target gene protein expression without affecting Nrf2 protein level. siRNA transfection procedure was described in 4D. Cells were then subject immunoblot analysis with indicated antibodies.

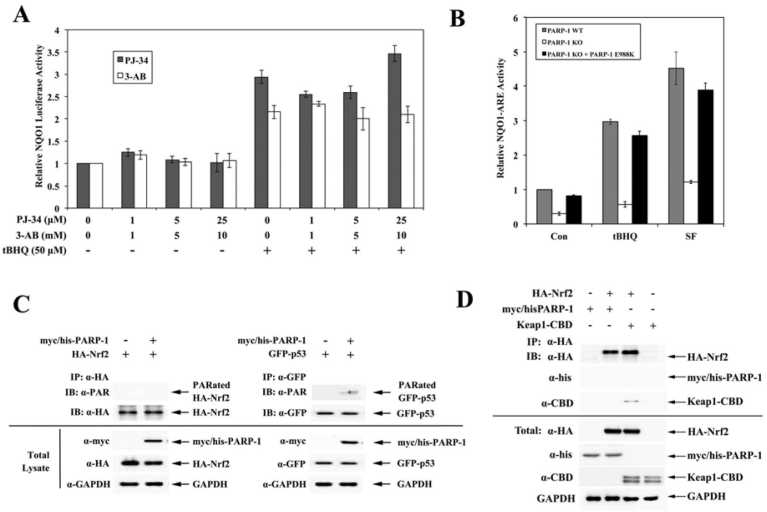

FIGURE 4.

Nrf2 is not a substrate for poly(ADP-ribosyl)ation, nor does PARP-1 associate with Nrf2. (A) Inhibition of PARP-1-mediated poly(ADP-ribosyl)ation does not affect transcriptional activity of Nrf2. MDA-MB-231 cells were co-transfected with expression vectors for NQO1-ARE-dependent firefly luciferase and TK-Renilla luciferase. Cells were left untreated or treated with the PARP-1 inhibitor PJ-34/3-AB alone or with both PJ-34/3-AB and tBHQ. Luciferase activities were measured. (B) PARP-1 enzymatic inactive mutant promotes Nrf2 transcriptional activity. PARP-$1^{-/-}$ cells were transfected with PARP-1-E988K as described in Figure 2D. (C) Nrf2 is not poly(ADP-ribosyl)ated. PARP-$1^{-/-}$ cells were transfected with indicated constructs for 48 h. Lysates were collected in RIPA buffer, followed by immunoprecipitation with an anti-HA or anti-GFP antibody. Poly(ADP-riobsyl)ated proteins were then detected using an anti-poly(ADP-ribose) antibody. (D) PARP-1 does not interact with Nrf2. HEK293 cells were transfected with the indicated constructs. Cell lysates were subjected to immunoprecipitation with HA-conjugated protein A beads and then analyzed by immunoblot.

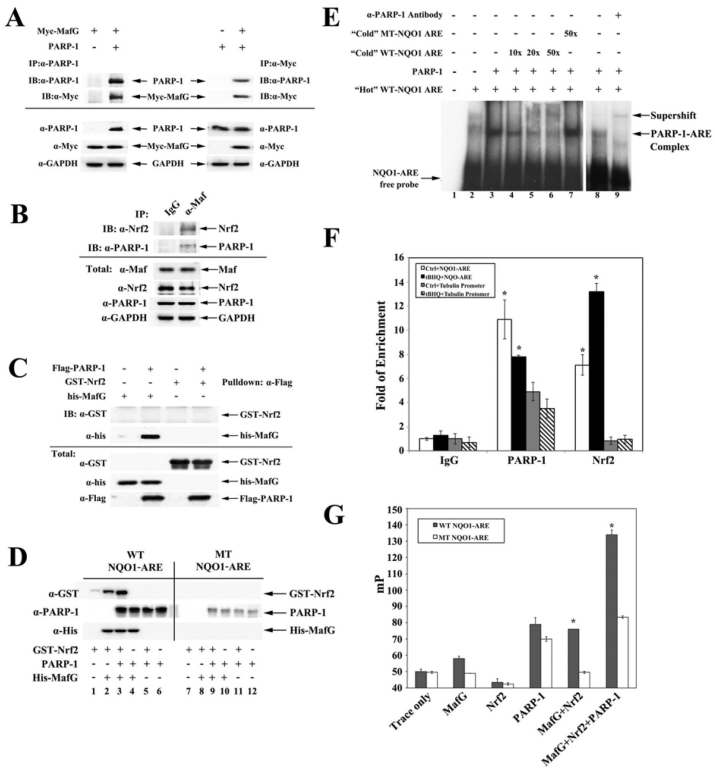

FIGURE 5.

PARP-1 interacts directly with small Maf protein and the ARE to enhance Nrf2 binding to the ARE. (A) Exdogenous PARP-1 and MafG interact reciprocally. PARP-1$^{-/-}$ cells were transfected with the indicated constructs. Immunoprecipitation assay was performed using either anti-PARP-1 or anti-myc antibodies. (B) Endogenous MafG immunoprecipitates PARP-1 and Nrf2. HEK293 cell lysates were collected in RIPA buffer containing 0.1% SDS, followed by immunoprecipitation with rabbit anti-Maf antibodies and immunoblotted with a mouse anti-PARP-1 antibody and a rabbit anti-Nrf2 antibody. (C) PARP-1 interacts directly with MafG but not Nrf2. Purified GST-Nrf2 and His-MafG were incubated with or without Flag-PARP-1. PARP-1-bound proteins were pulled down by Flag-M2 beads and subject to immunoblot by the indicated antibodies. (D) Interaction between Nrf2, MafG, PARP-1 and NQO1-ARE. Purified proteins were incubated with biotinylated NQO1-ARE DNA. ARE-bound proteins were pulled down by streptavidin beads and detected by immunoblot with the indicated antibodies. (E) PARP-1 binds directly to the NQO1 ARE. EMSA was performed using a radiolabeled NQO1-ARE oligonucleotide and the recombinant PARP-1 protein. (F) *In vivo* interaction of PARP-1 or Nrf2 with the ARE was determined by a ChIP assay. MDA-MB-231 cells were left untreated or treated with 50μM tBHQ for 16h. DNA-protein complexes were cross-linked and immunoprecipitated with the indicated antibodies or with IgG as a negative control. Amounts of DNA containing the NQO1-ARE or the tubulin promoter were semiquantified by real-time PCR amplification with a primer pair flanking the human NQO1 ARE sequence or a primer pair specific for the human tubulin promoter, and presented as a bar graph using the LightCycler 480 software. (G) PARP-1 enhances binding between Nrf2 and the ARE. Indicated proteins were incubated with fluorescent-tagged WT or MT human NQO1-ARE and fluorescence polarization was measured.

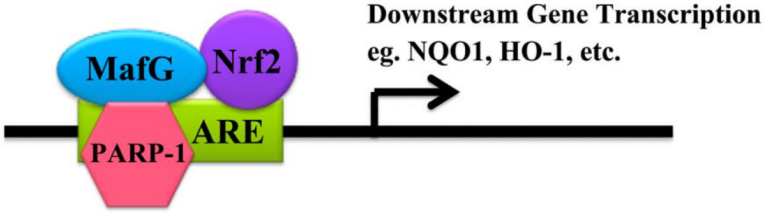

FIGURE 6.

Schematic model of Nrf2 regulation by PARP-1. PARP-1 binds directly to MafG and the ARE to enhance the interaction of Nrf2 with the ARE. Therefore, PARP-1 serves as a co-activator to upregulate the transcriptional activity of Nrf2, and thus the expression of Nrf2-target genes.