Regulation of Nrf2-ARE Signaling and Cellular Senescence by Jun Dimerization Factor 2

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1 Introduction

The environmental disruptors including xenobiotic, drugs, heavy metals and ionizing radiation can induce oxidative stress, which leads to the generation of reactive oxygen species (ROS) and electrophiles. ROS are key players on oxidative stress and generated as by-products of cellular metabolism, primary in the mitochondria. Oxidative stress is a state of an imbalance between pro-oxidant and anti-oxidant factors controlled by multiple components and might leads to the cellular damage because ROS can attack proteins, lipids and DNAs within cells. Eventually, this oxidative stress generates diseases such as cancer, cardiovascular complications, acute and chronic inflammation, and neurodegenerative diseases (Giudice et al., 2006). To prevent or delay the damages from ROS, cells possess anti-oxidant enzymes like superoxide dismutase MuSOD or Cu/ZnSOD, which are located in the mitochondria and in the cytoplasm, respectively, which they convert superoxide into hydrogen peroxide. The deposition of hydrogen peroxide to water and oxygen is further catalyzed by catalase. Another antioxidant defense mechanism includes non-enzymatic antioxidants such as glutathione (GSH), which functions in the cellular thiol/disulfide system. Therefore, it is obvious that cellular defense systems must labor to control constantly the levels of ROS and prevent their accumulation. Recently, the unexpected role of ROS on cellular senescence has been reported that the p16\textsuperscript{INK4a}–Rb pathway cooperated with mitogenic signals to enforce irreversible cellular senescence, and p16\textsuperscript{INK4a} protein regulated oxidative stress independent of both the Rb pathway and p16\textsuperscript{INK4a} mediated cell cycle control (Jenkins et al., 2011). Thus, the cell cycle modulators p21\textsuperscript{Cip1} (Macip et al., 2002) and p16\textsuperscript{INK4a} have recently emerged as important redox modulators.

Many antioxidant and/or detoxification enzymes, such as NADPH:quinone oxidoreductase 1 (NQO1) (Hou et al., 2001; Kasper et al., 2009), glutathione S-transferase (GST) (Rushmore et al., 1990), heme oxygenase-1 (HO-1) (Inamdar et al., 1996), and AP-1 transcription factor Fra-1 (Yoshioka et al., 1995), contribute to the cellular defense systems. Augmenting both the expression and the activity of phase II detoxification and antioxidant enzymes via the nuclear factor erythroid 2-related factor 2-anti-oxidant responsive element (Nrf2/ARE) core signaling pathways would be a rational approach for cancer chemoprevention and prevention of neural diseases, diabetes, muscle disorders, and inflammation (Giudice et al., 2006). Therefore, the protection mechanisms mediated by Nrf2-ARE are vital for cell growth, differentiation, and survival. Interestingly, these enzymes are often regulated coordinately through the core sequences located in their gene-regulatory regions, which are termed ARE/electrophile responsive element (ARE/EpRE) (Giudice et al., 2006), which also resembled with a musculo-aponeurotic fibrosarcoma (Maf) recognition element (MARE)-related sequence. Basic leucine zipper (bZIP) transcription factors, including the cap ‘n’ collar (CNC) protein family (Nrf1, Nrf2, Nrf3, and p45 NF-E2), form heterodimeric complexes with a small Maf protein (MafG, MafK, or MafF) and then bind ARE (Kasper et al. 2009). It is well established that the resulting Nrf2/small Maf complexes bind ARE to regulate the expression of genes that encode antioxidant and detoxification enzymes, thus preventing the accumulation of reactive oxygen species (ROS) and electrophiles (Rushmore et al., 1990; Motohashi et al., 2004; Katsuoka et al., 2005). Recently, Nrf2 was reported as a key molecule affecting stem cell renewal in both embryonic and adult tissues (Wakabayashi et al., 2010); it also assures mammalian longevity by stabilizing mitochondrial integrity (Kwon et al., 2012). The balance of ROS homeostasis and anti-oxidation response is critical for the cellular senescence.

The senescence response is essentially a DNA-damage response and can be induced by ionizing
radiation, replicative stress or activated oncogenes (Campisi, 2005; Campisi et al., 2007). It is also linked to a variety of morphological changes, such as an enlarged cell shape, decreased protein synthesis and degradation, resistance to apoptosis, increased activity of some lysosomal enzymes including senescence-associated β-galactosidase (SA-βgal) (Itahana et al., 2003), and the appearance of γ-H2AX, a marker of DNA damage (Herbig et al., 2006). Senescence-associated heterochromatin foci (SAHF) have been reported to play a role in the silencing of proliferation-promoting genes and thereby contribute to proliferation arrest (Narita et al., 2003). The chromatin is much more compact in cells exhibiting SAHF than in normal-interphase growing cells. SAHF contain several common markers of heterochromatin, including hyperacetylated histones, methylated H3K9, and bound HP1, but SAHP do not contain H3K10P, H2BS14P, or H3S28P. SAHP are also depleted of the linker histone H1 and are enriched in macroH2A and HMGA protein (Narita et al., 2003). Cellular senescence is an important barrier to the development of cancer in mammals due to its ability to arrest proliferation and prevent neoplastic progression of cells harboring oncogenic lesions (Campisi, 2005); however, accumulation of dysfunctional senescent cells contributes to mammalian aging (Campisi et al., 2007).

It has been well known that the Rb and p53 tumor suppressor pathways are one of master regulators of senescence. Inactivation of these two pathways abolished senescence in human and mouse (Wright et al., 2001). The expression of Rb and p53 is regulated by two distinct proteins, p16Ink4a and p14Arf (p19Arf in mouse), respectively, where are encoded by the cdkn2 locus. Although human cells lacking pRb and p53 circumvent senescence, most of these cells ultimately cease proliferating because of telomere shortening (Costanzi et al., 2001). The p53 pathway exerts its effects through activation of downstream target genes, including the cell cycle inhibitor p21Cip1, whose expression is increased in senescence cells.

This chapter will cover the role of the AP-1 chromatin modulator Jun dimerization protein 2 (JDP2) on antioxidant response and inhibition of ROS production, as well as the induction of replicative senescence.

2 Cellular Senescence and ROS Homeostasis

Cellular senescence involves in the processes that included three critical factors: (a) telomere shortening, (b) accumulation of DNA-damage, and (c) activation of p16Ink4a/p19Arf and p21Cip1. The contributions of these factors to senescence seem to differ from humans and mice (Sharpless et al., 1999). Cultured mouse fibroblasts underwent senescence even when they have long telomeres and high telomerase activity, and senescence was abolished by the Ink4a/Arf locus. In human cultured cells, the forced expression of telomerase is sufficient to overcome senescence by maintaining the telomere length (Gomez et al., 2012). These differences between humans and mice could be attributable to species specificity and/or experimental conditions. Takahashi et al. presented the unexpected role of ROS and cellular senescence; the p16Ink4a/Rb pathway cooperated with mitogenic signal to enforce irreversible cellular senescence (Takahashi et al., 2006). This mitogenic signal induced a growth-related production of ROS, and ROS levels accumulate to senescence-promoting levels through PKCδ. These data suggest that ROS participates in an anti-cancer mechanism against the well-defined tumor promoting activities of ROS (Ramsey et al., 2006). Chen et al. demonstrated that p21Cip1-dependent cell survival under oxidative stress is mediated through activation of the Nrf signaling pathway (Chen et al., 2009). This finding
present the key role for intracellular redox conditions in the p53–independent \( p21^{Cip1} \) gene regulation. Moreover, Jenkin et al. showed that \( p16^{INK4a} \) regulated oxidative stress independent of both the Rb pathway and \( p16^{INK4a} \)–mediated cell cycle control (Jenkins et al., 2011). In addition, it is well known that the telomere dysfunction initiated a DNA-damage response to induce the p53-dependent senescence and cell death, and ultimately may prevent tumorigenesis (Deng et al., 2008). It seems reasonable to assume that all three factors, activation of \( p16^{INK4a} \) and \( p21^{Cip1} \), telomere shorting, and accumulation of DNA damage have cooperative effects on aging in physiological situations. The coordination of these factors is critical for inducing the cellular senescence.

It is also known that the aging increased expression of JDP2 increases the expression of \( p16^{INK4a} \) and \( p19^{Arf} \) in normoxia condition, however, in the hypoxia this induction of JDP2 was lost (Nakade et al., 2009). The JDP2 is also induced by the oxidative stress and enhanced the anti-oxidation response and inhibited the ROS production (Yokoyama et al., 2012). To understand the molecular mechanism responsible for the direct transcriptional regulation of JDP2 by oxygen stress, we examined the response elements of the hypoxia response elements (HREs: G/ACGTG) in the promoter region of JDP2 and found that the basal luciferase activity of JDP2 promoter was lost under the hypoxia condition (Yokoyama et al., 2012). Alternatively, because the prolyl hydroxylase domain-containing enzymes (PHDs) are proposed “oxygen sensors” linking cellular oxygen concentration to HIF molecular responses, these sensor proteins such as PHD and FIH1 might associate with JDP2 as binding of oxygen. This character of oxygen-mediated gene expression is peculiar for JDP2.

3 Members of the bZIP Family

3.1 AP-1 Family

The Fos and Jun protein families include over 50 different molecules. These proteins participate in the regulation of a variety of cellular processes, which include the proliferation and differentiation of cells, apoptosis and oncogenesis (Grigoriadis et al., 1994). Members of this family (e.g., Fos, Fra-1, Fra-2, Fos B, c-Jun, JunB and JunD) are widely expressed in a variety of cells and tissues. The results of gene “knockout” and “knock in” experiments indicate that proteins in the Fos and Jun families have both overlapping functions and unique roles that cannot be deduced by the activities of other members of these families (Hai et al., 1989, Johnson et al., 1992; 1993; Wang et al., 1992; Schorpp-Kistener et al., 1999; Schreiber et al., 2000; Thepot et al., 2000). Proteins in the Fos and Jun families function as dimeric transcription factors that bind AP-1 regulatory elements in the promoter and enhancer regions of numerous mammalian genes (Kovany et al., 1992; Lallemand et al., 1997). Jun proteins not only form homodimers but they also form heterodimers with Fos proteins, whereas Fos proteins do not form homodimers and require heterodimerization to bind to DNA. The DNA-binding and dimerization domains of different members of the Fos and Jun families have similar DNA-binding and dimerization specificities. \( \text{In vitro} \), dimers formed by Fos and Jun bind with the highest affinity to the asymmetric heptanucleotide recognition sequences, TGA(C/G)TCA (AP-1 regulatory elements) and with slightly lower affinity to the symmetric octanucleotide TGACGTCA (CRE) (Landschnlz et al., 1988; Sonnenberg et al., 1989; MacCabe et al., 1993; Eckert et al., 1997). The AP-1 regulatory elements are ubiquitous regulatory elements that are found in a wide variety of promoter and enhancer regions. In natural promoter and enhancer regions, the sequence of the AP-1 regulatory elements often deviates
from the optimal recognition sequence. This variation in the recognition sequence may contribute to the
differential functions, at various regulatory elements, of different dimers of proteins in the Fos and Jun
families (Landschmlz et al., 1988). The functions of proteins in these two families depend on the specific
type of cell in which they are expressed. The activities and regulatory targets of these proteins are also
affected by specific signals that elicit the expression of 12-O-tetradecanoylphorbol 13-acetate-responsive
(TPA-responsive) and cAMP-responsive genes. Thus, the functions of proteins in the Fos and Jun
families are mediated by mechanisms that depend on the cellular context in which they are expressed.

The repertoire of Fos and Jun proteins within a given cell is subject to changes that occur in
response to various extracellular stimuli. Through dimerization, which is mediated by leucine zippers,
these proteins can form various homo- and heterodimers. The number of detectable Fos-Jun dimers
varies according to the type of cell (Kovany et al., 1992; Lallemand et al., 1997). Quiescent fibroblasts
in culture contain mainly homodimers of c-Jun and JunD; however, after stimulation of cells with serum,
heterodimers formed first by Fos and FosB and later by Fra-1 and c-Jun or JunB become the
predominant AP-1-binding proteins. In exponentially growing fibroblasts, heterodimers formed by Fra-2
and JunB or JunD are the predominant AP-1-binding complexes. In the mouse brain, metrazole-induced
seizures cause a similar sequence of association of components of different heterodimers, with the Fos
dimer being formed first. In undifferentiated osteoblasts in culture, all known members of the Fos and
Jun families are detectable; however, during differentiation, JunD, Fra-1, and Fra-2 become the
predominant AP-1-binding proteins. Similarly, during the differentiation of keratinocytes, the repertoire
of expressed members of the Fos and Jun families undergoes several temporally regulated shifts. This
progressive formation of different homo- and heterodimers that include proteins from the two families is
thought to contribute to the time-dependent induction of expression of various early and late genes in
different types of cells, in response to different types of stimuli.

### 3.2 Interactions between Proteins and other bZIP Families

Proteins in the Fos and Jun families are members of the bZIP family of transcription factors, which
contain a strongly conserved basic region that is involved in binding to DNA, and a heptad repeat of
leucine residues, the so-called leucine zipper, which is required for dimerization (Landschmlz et al.,
1988). All bZIP proteins form dimeric complexes via their leucine zippers, which juxtapose the two
basic regions of the proteins in the dimer to form a contiguous DNA contact interface at which each
monomer interacts with the major groove of one half sites (Ellenberger et al., 1992; Glover et al., 1995;
Fujii et al., 2000; Schmacher et al., 2000). Diversification of binding specificities through the formation
of inter-family dimers is a common characteristic of bZIP proteins. Inter-family dimerization has been
detected between proteins in the Fos and Jun families and various members of the group of ATF, C/EBP,
Maf, and NF-E2 (CNC) proteins. Several members of the ATF group of bZIP proteins form
heterodimers with proteins in the Fos and Jun families. The ATF-2 and ATF-3 proteins interact
preferentially with CRE (TGACGTCA) rather than with AP-1 elements. Heterodimerization of Fos and
Jun proteins and subsequent binding to CRE-like sites has been reported (Hai et al., 1991). Many of the
promoters that are controlled by heterodimers composed of ATF-2 and members of the Fos and Jun
families contain asymmetric binding sites (TTACCTCA in the Jun promoter and CTCAGTCA in the
adenovirus E2A promoter). Neither Fos-Jun heterodimers nor Jun-Jun homodimers bind efficiently to
these sites (Du et al., 1993; van Dam et al., 1993). As Jun and ATF proteins can be activated by different
protein kinases [Jun N-terminal kinase (JNK) and p38 stress-activated protein kinase (SAPK),
respectively], dimerization of members of the Jun family with members of the ATF family might be expected to mediate the integration of signals from different signal-transduction pathways (van Dam et al., 1998; Gozdecka & Breitwieser, 2012).

4 Factors for Regulation of Antioxidant Response Element (ARE)

4.1 Regulators of ARE

ARE–regulatory transcription factors have been identified including c-Jun, Jun-B, Jun-D, c-Fos, Fra1, Nrf1, Nrf2, ATF3 and ATF4 bind to the AREs in genes of various cytoprotective enzyme genes (Table 1). Furthermore, Nrf2 heterodimerizes with c-Jun, JunB, and JunD proteins that binds to the ARE resulting in expression and coordinated induction of detoxifying enzyme genes in response to antioxidants and xenobiotics (Venugopal et al., 1998). Nrf2 belongs to the CNC family composed of four related transcription factors, p45 NF-E2, Nrf1, Nrf3, Bach1 and Bach2. The small Mafs have been shown to be critical for the CNC factor binding to MARE. ARE core sequence is identical to either the tumor-promoter response element (TRE) or cyclic AMP response element (CRE). Previous studies have been shown that the small Maf proteins recognize GC sequences in consensus ARE element, whereas the CNC proteins bind to the core sequence (Yamamoto et al., 2006; Kurokawa et al., 2009). A human transcription factor hMAF was shown to homodimerize and heterodimerize with Nrf1 and Nrf2 (Marini et al., 1997). Although hMAF homodimers and hMAF/Nrf1 and hMAF/Nrf2 heterodimers bind to the similar ARE core site, they possess functionally opposite effects. The hMAF homodimers repress β-globin gene expression, whereas hMAF heterodimerization with Nrf1 and Nrf2 activates β-globin gene expression. Nrf2/MafK heterodimer has shown to bind to the ARE of a gene encoding a subunit of GST-Ya, suggesting that the Nrf2/MafK heterodimer may play a role in the ARE-mediated induction of GST Ya gene expression (Itoh et al., 1997). Consistent with this results, knockout of all small Maf proteins in MEF cells showed reduction of Nrf2-regulatory gene expressions including ferritin heavy (Fth1), glutamate-cysteine ligase catalytic (GCLC), Gsta4, heme oxygenase 1 (HO-1), thioredoxin reductase (Txnrd1) and NQO1. However, overexpression of MafK suppressed of ARE-mediated transcription in biochemical studies (Nguyen et al., 2000). The negative regulation of ARE-mediated expression has been observed in c-Fos KO mice. C-Fos KO mice showed increased expression of NQO1 and GST Ya as compared to wild-type and c-Fos KO mice (Wilkinson et al., 1998). Moreover, overexpression of c-Fos and Fra1 in transfected HepG2 cells repressed the ARE-mediated gene expression (Venugopal et al., 1996). Furthermore, Bach1 also well characterized as negative repressor of ARE-mediated HO-1 gene expression. Bach1 heterodimerization with small Maf proteins strongly suppressed HO-1 expression. Currently, we showed that JDP2 positively regulate HO-1 expression and required for activity of ARE activity (Yokoyama et al., 2012). Furthermore, JDP2 interacted with MafK and Nrf2, and bind to ARE as a regulator of antioxidant enzymes. Therefore, it appears that ARE-mediated expression of detoxifying enzyme genes is balanced by positive and negative regulatory factors.
### Table 1: Transcription factors which are related with the ARE regulation.

<table>
<thead>
<tr>
<th>Transcription factor</th>
<th>ARE regulation</th>
<th>Interaction with Nrf2</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>ATF3</td>
<td>Yes</td>
<td>Down</td>
<td>Brown et al., 2008; Kim et al., 2010</td>
</tr>
<tr>
<td>ATF4</td>
<td>Yes</td>
<td>Up</td>
<td>He et al., 2001</td>
</tr>
<tr>
<td>Bach1</td>
<td>Yes</td>
<td>Down</td>
<td>Dhakshinamoorthy et al., 2005; Sun et al., 2002</td>
</tr>
<tr>
<td>Bach2</td>
<td>Yes</td>
<td>Down</td>
<td>Muto et al., 2001</td>
</tr>
<tr>
<td>c-Jun</td>
<td>Yes</td>
<td>Up</td>
<td>Venugopal et al., 1998</td>
</tr>
<tr>
<td>c-Fos</td>
<td>Yes</td>
<td>Down</td>
<td>Venugopal et al., 1996; Venugopal et al., 1998</td>
</tr>
<tr>
<td>Fra-1</td>
<td>ND</td>
<td>Down</td>
<td>Venugopal et al., 1996</td>
</tr>
<tr>
<td>Fra-2</td>
<td>ND</td>
<td>Down</td>
<td>Venugopal et al., 1996</td>
</tr>
<tr>
<td>JunD</td>
<td>Yes</td>
<td>Up</td>
<td>Venugopal et al., 1998; Tsuji et al., 2005; Yeligar et al., 2010</td>
</tr>
<tr>
<td>JunB</td>
<td>Yes</td>
<td>Up</td>
<td>Venugopal et al., 1998; Yeligar et al., 2010</td>
</tr>
<tr>
<td>MafF</td>
<td>Yes</td>
<td>Down</td>
<td>Motohashi et al., 2004</td>
</tr>
<tr>
<td>MafG</td>
<td>Yes</td>
<td>Down</td>
<td>Itoh et al., 1997</td>
</tr>
<tr>
<td>MafK</td>
<td>Yes</td>
<td>Down</td>
<td>Itoh et al., 1997</td>
</tr>
<tr>
<td>Nrf1 with small Maf</td>
<td>Down</td>
<td>ND</td>
<td>Venugopal et al., 1996; Zhang et al., 2006</td>
</tr>
<tr>
<td>Nrf2 with small Maf</td>
<td>Up</td>
<td>-</td>
<td>Itoh et al., 1997</td>
</tr>
<tr>
<td>Nrf3 with small Maf</td>
<td>Down</td>
<td>ND</td>
<td>Sankaranarayanan et al., 2004</td>
</tr>
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</table>

### 4.2 Modulation of Nrf2-mediated ARE Activation by Other Nuclear Factors

It has been demonstrated that Nrf2 required dimerization partner for ARE binding, while it contains bZIP DNA binding domain (Itoh et al., 1997). Thereby, small Maf proteins are well accepted partners to form dimerization with Nrf2 for ARE-mediated gene expression. However, small Maf proteins contain bZIP domain to bind DNA, but no transactivation domain, and have strong repressor activity of transcription on ARE domain, suggesting possibility that Nrf2/MafK complex required for other binding factors for full activity of ARE-mediated transcription. Previous studies have shown that the Neh4 and Neh5 domains of Nrf2 interact with cAMP response element-binding protein (CREB)-binding protein (CBP), which possesses histone acetyltransferase activity (Katoh et al., 2001). Zhang et al., have shown that Nrf2 recruits Brahma-related gene 1 (BRG1), a chromatin remodeling factor to the HO-1 and NQO1 promoter on the Neh4 and Neh5 domains of Nrf2 and led to enhanced binding of RNA polymerase II and increased transcription (Zhang et al., 2006). Therefore, CBP serves as a co-transcription activator of Nrf2, allowing chromatin to relax promoters including ARE, thereby facilitate the recruitment of RNA polymerase II. Since, MafK and JDP2 interact with HDAC3 (Jin et al., 2002; Liu et al., 2008), it is possibility that MafK interact with HDAC3 in absence of JDP2 there by exerts inhibitory effect on ARE transcription. In the presence of JDP2, MafK preferentially interact with JDP2, and then HDAC3 dissociated from MafK/ARE nuclear complex, Nrf2 interacts with CBP occurring following this process to activate ARE-mediated transcription.
4.3 Cytoprotection by Antioxidant Responsive Element (ARE)

Cytoprotection mediated by cellular defense system such as antioxidant enzymes and phase II detoxification enzymes, which regulated by ARE in their promoter regions. ARE was initially identified as EpRE in the promoter region of mouse glutathione S-transferase alpha 1 (Gsta1) gene, can be activated by β-naphthoflavone (β-NF), tert-butyl-1, 4-hydroquinone (tBHQ) as well as di-phenos that can mimic redox-cycle (Friling et al., 1990). Following deletion and mutation analysis of a 41 bp region in the promoter of human glutathione S-transferase A2 (GSTA2) gene showed the core enhancer sequence (5'–TGACNNNGC–3') is critical for ARE. Similar elements that could respond to β-NF and tBHQ were found in the promoters of rat and human NQO1 genes (Favreau et al., 1991). In addition to core element of ARE, Wasserman et al. proposed that the fully functional enhancer element from aliment analysis among rodent Gsta1 or Gsta2, rat NQO1, human NQO1 to be extended 20 bp as ‘consensus ARE’ (5'–TMANNRGTGAYNNNGC–3’) (Wasserman et al., 1997). Intriguingly, genes encoded several antioxidant enzymes (glutamate-cysteine ligase catalytic; GCLC, thioredoxin; TRX, and sulfiredoxin; SRXN1), detoxification proteins (aldo-keto reductase 1C2; AKR1C2, glutathione S-transferase P1; GSTP1, and NQO1) and metal-binding proteins (ferritin heavy; Fth1, ferritin light; FTL, and metallothionein; Mt2) contained AP-1 element at ‘NNN’ in ARE core sequence with palindrome motif, suggesting that ARE/AP1 element has capable of recruiting homodimers or heterodimers of Jun and Fos proteins (Hays et al., 2010). Therefore, it has been considered that redundant ARE motifs can be response to different extracellular stimuli, consensus ARE may response to electrophiles and chemopreventive agents via the TMANNR plus core ARE site. On the other hand, environmental stressors such as UV radiation and 12-O-tetra-decanoylphorbol-13-acetate (TPA) activate ARE via the AP1 site (‘NNN’) (Angel et al., 1987).

4.4 Canonical Nrf2-ARE Pathway

Nrf2, a member of the CNC family of bZIP proteins, is extensively proven to be a strong activator of ARE-mediated gene expression (Wasserman et al., 1997). Nrf2 knockout mice lacking the expression of Nrf2 showed significantly reduced expression and induction of ARE-driven gene expression, indicating a role of Nrf2 in the regulation of antioxidant enzymes (Itoh et al., 1997; McMahon et al., 2001). Under the homeostatic conditions, Nrf2 is sequestered in the cytoplasm, where it is associated with kelch-like ECH-associated protein1 (Keap1), an actin-binding protein (Hays et al., 2001). The presence of a stimulus leads to the disruption of the Keap1/Nrf2 complex, nuclear translocation of Nrf2, and binding to the ARE with small Maf proteins (Itoh et al., 1997). Keap1 contains a bric-a-bric, tramtrack, broad complex (BTB) domain and a C-terminal kelch repeat domain, which binds to the Neh2 domain of Nrf2. Therefore, Keap1 retains Nrf2 in the cytoplasm to repress the Nrf2-mediated ARE activity (Nrf2-ARE). It has been demonstrated that the Keap1-dependent ubiquitination of Nrf2 functions constitutively to degrade Nrf2 under homeostatic conditions (McMahon et al., 2003). Ubiquitination of Nrf2 is provided by the Cul3-Rbx1 E3 ubiquitin ligase complex, which binds to the N-terminal BTB domain and central linker region of Keap1 (Zhang et al., 2004; Kobayashi et al., 2005). Previous studies suggested that ubiquitination of Keap1 was markedly increased in cells exposed to quinone-induced oxidative stress, in parallel with inhibition of Keap1-dependent ubiquitination of Nrf2, and resulted in decreased steady-state levels of Keap1, which degraded by a 26S proteasome-independent system (Zhang et al., 2004; 2005). Therefore, Keap1 considered to acts as a sensor of oxidative stress. Since, Nrf2 is a labile protein,
stabilizing Nrf2 is considered to be important to maintain the cellular defense system, which is dependent on the status of the Nrf2-Keap1 complex.

5 Inhibition of Histone Modification by JDP2

SOS recruitment screening system and yeast two-hybrid screening were used to identify Jun dimerization protein 2 (Aronheim et al., 1997; Jin et al., 2001). JDP2 forms heterodimers with c-Jun and ATF-2 represses the activation protein 1 (AP-1)-mediated activation of transcription. JDP2 was also shown to associate with both the CAAT/enhancer-binding protein-γ (Broder et al., 1998) and the progesterone receptor and the glucocorticoid receptor (Wardell et al., 2002; Hill et al., 2009; Garza et al., 2011). JDP2 is constitutively expressed in many cell lines and represses the transcriptional activity of AP-1 (Pan et al., 2010), but can potentiate transcription with associates with CHOP 10 (Weidenfeld-Baranboim et al., 2008). JDP2 inhibits UV-induced apoptosis by suppressing the transcription of the p53 gene (Piu et al., 2001). Given the roles of AP-1 in cellular transformation and the reported repression of Jun- and ATF-2-mediated transcription by JDP2, it was demonstrated that JDP2 inhibits the oncogenic transformation of chicken embryonic fibroblasts at some extent (Blazek et al., 2003). JDP2 also modulates the expression of cycling D1, cyclin A2 and p21Cip1, which have opposing effects on cell cycle progression (Ostrovsky et al., 2002; Heinrich et al., 2004; Pan et al., 2010). JDP2 interfered with the progression of the cell cycle by reducing the levels of cyclin D1 and at the same time, increases the expression of p21Cip1. The forced expression of JDP2 promotes the myogenic differentiation of C2C12 cells, which is accompanied by the formation of C2 myotubes and the strong expression of major myogenic markers. The ectopic expression of JDP2 in rhabdomyosarcoma cells induces incomplete myogenesis and the incomplete formation of myotubes. These cells become committed to differentiation via the p38-MAPK pathway (Ostrovsky et al., 2002). A similar enhancement of cell differentiation was reported during the induction of osteoclast formation by the receptor activator of the nuclear factor κB-ligand, RANKL (Kawaide et al., 2003). The JDP2 controlled the bone homeostasis and antibacterial immunity by regulating osteoclast and neutrophil differentiation (Maruyama et al., 2012). The levels of JDP2 remain constant in response to a large variety of stimuli, such as UV, irradiation, and retinoic acid (RA), which affect the levels of other factors involved in cell-cycle control. The induction of JDP2 expression is observed only during the differentiation of F9 cells, muscle cells and osteoclasts. JDP2 may provide a threshold for exit from the cell cycle and a commitment to differentiation. It is also interesting that JDP2 is one candidate oncoprotein that collaborates in the oncogenesis associated with the loss of p27 as the result of insertion mutations (Hwang et al., 2002; Stewart et al., 2007). Recent studies of tumor cells have demonstrated that JDP2 is a tumor suppressor (Heinrich et al., 2004) but also can promote hepatocellular transformation in transgenic mice with liver specific JDP2 expression (Bitten-Worm et al., 2010). We have reported previously that acetylation by p300 is inhibited in a dose-dependent manner by exogenous JDP2 (Jin et al., 2006). An inhibitory effect of JDP2 was detected on histone acetylation induced by p300, CREB-binding protein (CBP), p300/CBP-associated factor (PCAF), and general control nonrepressive 5 (GCN5). The overexpression of JDP2 apparently represses the RA-induced acetylation of lysines 8 and 16 of histone H4 and some amino terminal lysine residues of histone H3. The template activation factor-1β protein (TAF-Iβ protein), a component of the inhibition of histone acetylase complex (INHAT complex) identified by Seo et al. (Seo et al., 2001; 2002), is a histone chaperone that binds
directly to core histones and facilitates the assembly of nucleosomes in vitro. JDP2 interacted directly with all the core histones tested and inhibited the p300-mediated acetylation of those histones. To our surprise, JDP2 also introduced supercoils into circular DNA in the presence of core histones, to levels similar to those observed for yeast CCG1-interacting factor 1 protein (yCia1p) and CCG1-interacting factor (CIA1). Therefore, JDP2 appears to have significant histone chaperone activity in vitro (Jin et al., 2006). We have also shown that the HAT-inhibitory activity of JDP2 is involved, to some extent, in the repression of transcription by JDP2, whereas the maximal capacity of JDP2 to suppress the RA-mediated activation of the c-Jun promoter (Jin et al., 2002) and to suppress the adipocyte differentiation (Nakade et al., 2007) requires the indirect recruitment of histone deacetylases (HDACs).

6 Mechanism of JDP2-mediated Regulation of p16\textsuperscript{Ink4a}/p19\textsuperscript{Arf} for Senescence

We showed that the expression of JDP2 regulates the senescence-competent genes, p16\textsuperscript{Ink4a} and p19\textsuperscript{Arf}, in response to accumulating oxidative stresses (Nakade et al., 2009). The Jdp2\textsuperscript{−/−} MEFs continued to divide, even after six weeks, whereas the wild type (WT) MEFs almost stopped proliferating and entered senescence under environmental oxygen. Conversely, both WT MEFs and Jdp2\textsuperscript{−/−} MEFs did not succumb to replicative senescence at hypoxia condition (Nakade et al., 2009). These results demonstrate that MEFs lacking JDP2 can escape from the irreversible growth arrest caused by environmental oxygen. The expressions of p16\textsuperscript{Ink4a} and Arf were repressed in aged Jdp2\textsuperscript{−/−} MEFs (40 days) compared with their levels in wild-type MEFs. In 3% oxygen, at the equivalent time (40 days), wild-type MEFs expressed lower levels of p16\textsuperscript{Ink4a} and Arf compared with those grown in 20% oxygen, whereas Jdp2\textsuperscript{−/−} MEFs maintained low-level expression of p16\textsuperscript{Ink4a} and p19\textsuperscript{Arf}. Thus, these data indicate that the aging-associated expression of p16\textsuperscript{Ink4a} and p19\textsuperscript{Arf} is dependent on oxygen stress and that JDP2 controls the expression of both p16\textsuperscript{Ink4a} and p19\textsuperscript{Arf}. We found no dramatic down regulation of the upstream repressors of p16\textsuperscript{Ink4a}/p19\textsuperscript{Arf}, Bmi1, and Ezh2, in the absence of JDP2, suggesting that JDP2 does not regulate their expression. Interestingly, JDP2 expression in wild-type MEFs increased in the presence of 20% oxygen but not in the presence of 3% oxygen (hypoxia condition), suggesting that its expression depends on oxygenic stress and that accumulated JDP2 may play a role in the transcriptional activation of p16\textsuperscript{Ink4a}/p19\textsuperscript{Arf}. Studies based on the ChIP assay demonstrated that the methylation of H3K27 at the p16\textsuperscript{Ink4a}/p19\textsuperscript{Arf} locus is greater in Jdp2\textsuperscript{−/−} MEFs than in wild-type MEFs. The binding of PRC1 and PRC2 to the p16\textsuperscript{Ink4a} and p19\textsuperscript{Arf} promoters is more efficient in Jdp2\textsuperscript{−/−} MEFs than in wild-type MEFs. These observations suggest that, in the absence of JDP2, H3K27 is methylated by PRC2 and the p16\textsuperscript{Ink4a}/p19\textsuperscript{Arf} locus is silenced by PRC1, whereas the increased expression of JDP2 helps to release PRC1 and PRC2 from the p16\textsuperscript{Ink4a}/p19\textsuperscript{Arf} locus, thereby reducing H3K27 methylation (Figure 1). We conclude that JDP2 is an important factor regulating cellular senescence. The loss of JDP2 allows MEFs to escape senescence and, conversely, the overexpression of JDP2 induces cell-cycle arrest. The absence of JDP2 reduces the expression of both p16\textsuperscript{Ink4a} and Arf, which inhibit cell-cycle progression.
Figure 1: Proposed model of the epigenetic regulation of the expression of the genes for p16 Ink4a and p19 Arf by JDP2. The exposure of young MEF primary cells to aging stress leads to the accumulation of JDP2. JDP2 binds to histones and inhibits the methylation of H3K27 at the p16 Ink4a/p19 Arf locus. As a result, PRC1 and PRC2 fail to form stable repressive complexes and are released from the locus. The consequent expression of p16 Ink4a and p19 Arf in the aged cells leads to growth arrest and senescence stage (Nakade et al. 2009).

7 Regulation of Nrf2-MafK Complex by JDP2 Cofactor

The level of ROS is tightly controlled by an inducible antioxidant program that responds to cellular stressors and is regulated predominantly by Nrf2 and its repressor protein, the kelch-like ECH-associated protein 1 (Keap 1) (Motohashi et al., 2002; Newman et al., 2003; Tanigawa et al., 2007). In contrast to the acute response of Nrf2, in the steady state, some somatic mutations cause destabilization of Nrf2 and decrease the constitutive transcription of its target genes, indicating that enhanced ROS detoxification and additional Nrf2 functions may be critical for the induction of the antioxidant response (Hayes et al., 2009). Because JDP2 is a member of the stress-induced AP-1 protein family (Aronheim et al., 1997), we examined the role of JDP2 in cell proliferation, ROS production, and antioxidant response and then identified the JDP2 transcription factor as a cofactor that enhances the ARE activity. JDP2 bound to ARE and regulated the ARE-mediated transcription associated with the Nrf2/MafK factors. The Nrf2 is known as a central regulator of the induction of many antioxidant-responsive genes and genes encoding phase II detoxification enzymes. However Nrf2 is not DNA-binding protein and the addition of Nrf2 and MafK
lead to the repression of ARE reporter genes. Thus, the real target molecule to enhance the ARE response in response to the oxidative stress remains to be identified. Thus we implicate that JDP2 is one of such molecules to enhance the transcription activity of ARE reporter genes and to inhibit ROS production to form the positive complex with Nrf2/MafK via leucine zipper domains. Therefore, JDP2 acts not only as an AP-1 repressor protein, to suppress cell proliferation during cancer progression, but also participates in maintenance of ROS homeostasis to prevent cell damage by ROS.

8 Discussion

Histone acetyltransferase or histone methyltransferase may not have access to the nucleosome in vitro because JDP2 binds to not only the specific AP-1 site but also the core histone or nucleosome partially in a DNA-sequence-specific manner or histone subset-specific manner. The ChIP assay demonstrated that JDP2 inhibits at least the acetylation of histones H4K8 and H4K16, although we have not determined other precise residues of histone H3 acetylation (Jin et al., 2006). Moreover, JDP2 associates with histone H3K27 and blocks the methylation of histones (Nakade et al., 2009). Thus, we assume that the interaction of JDP2 with the nucleosome is specific to the DNA sequence or histone modification, and thus only certain restricted sets of histones might be associated with JDP2 in vivo. Addressing these precise functions in the context of epigenesis will help us understand the regulation of senescence, differentiation and viral infection in a broader context. In the presence of antioxidant, JDP2 is a cofactor that reinforces the formation of the possible triple complex and then activates the antioxidant response element (ARE; Yokoyama et al., 2012). It is also possible that JDP2 might help to stimulate the ARE activity of MafK/Nrf2 to convert the repressor function of this duplex to the active stimulatory function. JDP2 was also critical for the inhibition of generating ROS and DNA oxidation and induced cellular response to oxidative stress. Understanding how JDP2 promotes ARE and senescence, whether by inducing cellular senescence or decreasing the frequency of cell-cycle entry, is an important issue. How the antioxidant induces the gene expression of JDP2 is also interesting. In the induction of cellular senescence by JDP2, we propose a model: that the aging upregulates JDP2 expression in primary untransformed cells. Increased JDP2 helps to remove PRC1 and PRC2, which are responsible for the methylation of histone H3, from the $p16^{\text{ink4a}}$/Arf locus, leading to increased $p16^{\text{ink4a}}$ and Arf expression and entry into senescence. Our findings provide new insights into the molecular mechanisms, by which senescence is induced in the context of the epigenetic regulation of the $p16^{\text{ink4a}}$/Arf locus (Figure 1). There is a balance between ARE and ROS. The antioxidant reagents or the oxidative stress induced the expression of JDP2 and then form the triple complex to stimulate the ARE, conversely, JDP2 inhibits the ROS production to reduce the response to the oxidative stress (Figure 2). In similar, Jenkins et al. (2011) reported that the loss of $p16^{\text{ink4a}}$ increased the intracellular level of ROS, which can be lost upon the re-expression of $p16^{\text{ink4a}}$. This novel alternative Rb-independent regulation of $p16^{\text{ink4a}}$ might be functional in the case of JDP2. The JDP2 also stimulates the expression of $\text{INK4a/ARF}$ locus to exclude the polycomb complex PRC1 and PRC2 for the $\text{INK4a/ARF}$ loci. This coordination of activations of ARE and $\text{INK4a/ARF}$ locus induced by JDP2 results the cellular senescence and protection of ROS production to maintain ROS/ARE homeostasis. These findings suggest that JDP2 plays a potential role in protecting cells against the malicious attack of carcinogens and endogenous reactive oxygen/nitrogen species, by inducing several detoxifying/antioxidant enzymes.
Figure 2: Schematic model of the interrelationship between ARE complex and ROS generation. The molecular relationship of ARE complex and ROS production are presented to explain the maintenance of ROS homeostasis (Yokoyama et al., 2012).

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