Review

Unique and overlapping roles of NRF2 and NRF1 in transcriptional regulation

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Transcription is regulated by specific transcription factors that mediate signaling in response to extrinsic and intrinsic stimuli such as nutrients, hormones, and oxidative stresses. Many transcription factors are grouped based on their highly conserved DNA binding domains. Consequently, transcription factors within the same family often exhibit functional redundancy and compensation. NRF2 (NFE2L2) and NRF1 (NFE2L1) belong to the CNC family transcription factors, which are responsible for various stress responses. Although their DNA binding properties are strikingly similar, NRF2 and NRF1 are recognized to play distinct roles in a cell by mediating responses to oxidative stress and proteotoxic stress, respectively. In this review, we here overview the distinct and shared roles of NRF2 and NRF1 in the transcriptional regulation of target genes, with a particular focus on the nuclear protein binding partners associated with each factor.

***Key Words*:** **transcription, protein complex, oxidative stress, ER stress**

Nuclear factor erythroid 2-related factor 2 (NFE2L2/NRF2) is a transcription factor that activates genes involved in the oxidative stress response, such as *Nqo1*, *Gclm*, and *Gclc*, to protect cellular components from damage caused by reactive oxygen species (ROS).[1] Recent reports have also shown that NRF2 regulates cellular metabolism by activating genes such as the pentose phosphate pathway.[2–4] Under normal conditions, NRF2 is targeted for degradation by the Kelch-like ECH-associated protein 1 (KEAP1) E3 ligase-dependent proteasome pathway.[5,6] However, when exposed to oxidative stress, the activity of KEAP1 E3 ligase is suppressed, resulting in NRF2 stabilization, nuclear translocation, heterodimerization with small Maf (sMaf) proteins, and binding to the antioxidant response element (ARE) DNA sequences of target genes.[1,7] Some cancers possess loss-of-function mutation of *KEAP1*, leading to constitutive activation of NRF2.[8–13] The target gene activation by NRF2 is context dependent, especially in NRF2-activated cancer cells, NRF2 activates the gene expression of NOTCH3, a gene required for promotion of tumor-initiating activity.[14]

Nuclear factor erythroid 2-related factor 1 (NFE2L1/NRF1) is a transcription factor responsible for orchestrating the "proteasome bounce-back response" which is the cellular transcriptional activation of a series of proteasome subunit genes to restore proteasome activity after its suppression.[15,16] Under normal conditions, NRF1 is also subjected to degradation by proteasome machinery.[15] Unlike NRF2, NRF1 is localized in the endoplasmic reticulum (ER).[17–22] When cells are exposed to ER stress, such as exposure to proteasome inhibitors, the C-terminal portion of NRF1 is retrotranslocated from the ER lumen to the cytoplasm, facilitated by the ATP-driven chaperone valosin-containing protein (VCP/p97).[23] Subsequently, NRF1 undergoes de-*N*-glycosylation, a process mediated by *N*-glycanase 1 (NGLY1).[24] The de-*N*-glycosylated NRF1 is then cleaved by DNA damage-inducible 1 homolog 2 (DDI2). The processed NRF1 is translocated into the nucleus, where it forms a heterodimer with sMAF, enabling it to bind to ARE sequences and activates target genes, including proteasome subunits genes.[25–28] NRF1 also functions as a cholesterol sensor, a role attributed to its ER localization.[29]

As NRF2 and NRF1 domains required for DNA binding and heterodimerization are well conserved, their DNA binding features are similar in *in vitro* assay.[30,31] In this review, we describe the common and different features in domain structures, target genes, and transcriptional roles of NRF2 and NRF1. Additionally, we highlight the differences in nuclear interacting partner proteins between NRF2 and NRF1.

Domain Structure

NRF2 and NRF1 both possess seven conserved domains, referred to as NRF2-ECH homology (Neh) 1–7 domains (Fig. 1).[32] These domains can be grouped into two categories: common domains (Neh1 and Neh6) and unique domains (Neh2–5 and Neh7). The Neh1 domain consists of the Cap'n'Collar (CNC) region and basic region-leucine zipper (bZip) motif, mediating heterodimerization with sMAF proteins and DNA binding at ARE sequences. Neh6 is recognized by the beta-transducin repeat-containing protein (βTrCP)-Cullin 1 (CUL1) E3 ubiquitin ligase complex and functions as a degron.[33–35]

Neh2 is essential for the degradation of NRF2 and contains two motifs known as DLG and ETGE, which enable interaction with KEAP1.[1] KEAP1 is a substrate recognition subunit of Cullin 3 (CUL3)-based E3 ubiquitin ligase and serves as a sensor for electrophiles. While NRF1 also possesses completely conserved DLG and ETGE motifs within Neh2, mutations in these motifs do not result in any observable changes in transcriptional activity or protein levels of NRF1.[17] This indicates that the functional contribution of Neh2 domains differs between NRF2 and NRF1. Neh3, Neh4/5, and Neh7 domains of NRF2 serve as transcriptional regulation domains.[36–45] In particular, Neh4/5 exhibits strong transcriptional activity due to its interaction with various transcriptional activators.[36,40,41] Neh7 is reported as a transcriptional repression domain through its binding with RXRα.[44,45] Neh3, Neh4/5, and Neh7 domains of NRF1 are also expected to function as transcriptional regulation domains; however, detailed analysis in this point remains to be done.[46] In addition to the Neh1–7 domains, NRF1 possesses a unique N-terminal extensive domain that binds to ER, conferring responsiveness to ER stress on NRF1.[19]

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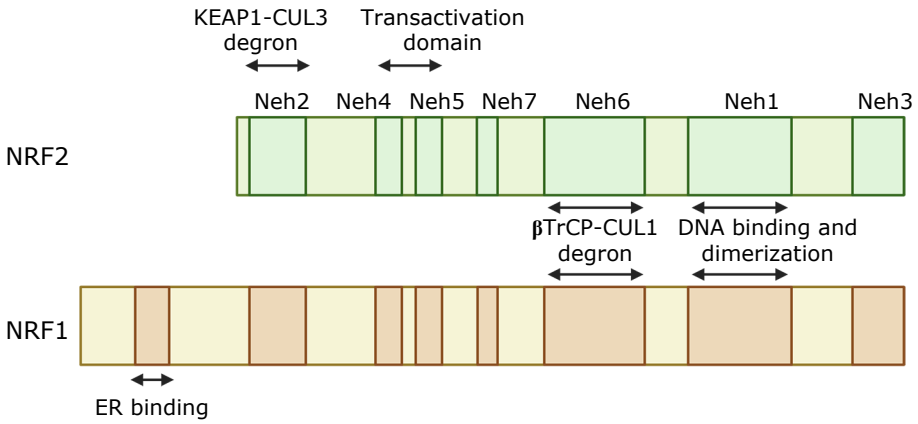

Fig. 1. Domain structures of NRF2 and NRF1. NRF2 and NRF1 share two functionally conserved domains: Neh6, responsible for β-TrCP-dependent proteasomal degradation, and Neh1, involved in DNA binding and heterodimerization with sMAF. NRF2 possesses unique domains: Neh3, Neh4/5, and Neh7, which are related to transcriptional regulation, and Neh2, which is crucial for Keap1-dependent proteasomal degradation. NRF1 contains a distinctive *N*-terminal domain responsible for endoplasmic reticulum (ER) binding.

Unique and Overlapping Roles of NRF2 and NRF1 in Transcriptional Regulation

NRF2 and NRF1 were initially identified as NF-E2-related transcription factors, sharing highly conserved CNC and bZIP domains.[47,48] Although the DNA binding motifs of NRF2 and NRF1 exhibit striking similarity, the generation of gene knockout mice unveiled notable functional distinctions between NRF2 and NRF1. *Nrf2*-null mice display no apparent phenotypic alterations[49] but demonstrate a decrease in inducible gene expressions in response to oxidative stress.[50] In contrast, *Nrf1*-null mice are embryonically lethal, likely due to impaired fetal liver erythropoiesis.[51] While *Nrf1*-null embryos survive until embryonic day 13.5, *Nrf1*:*Nrf2* double-knockout embryos were not observed at this stage, suggesting functional redundancy and compensation between NRF1 and NRF2 in early mouse development.[52] Interestingly, mouse embryonic fibroblasts (MEFs) lacking both *Nrf1* and *Nrf2* exhibit increased intracellular ROS and cell death compared to single knockout MEFs under normal oxygen conditions.[52] However, low oxygen (5% O_2) culturing dramatically reduces cell death of double knockout MEFs.[52] These results indicate a redundant and compensatory relationship between NRF1 and NRF2 in regulating cellular ROS levels.

NRF1 has been reported to comprehensively activate proteasome subunit genes through binding to AREs in response to proteasome inhibition.[15,16] Proper proteasome activity is essential for maintaining normal neural function because defects in its activity can lead to neurodegenerative diseases.[53] Knockout mice with brain- or central nervous system-specific NRF1 deficiencies impair proteasome function, resulting in the aberrant accumulation of ubiquitinated proteins and subsequent neurodegeneration.[54,55]

It was reported that NRF2 induces the expression of some proteasome subunit genes in response to electrophiles, such as sulforaphane and 3*H*-1,2-dithiole-3-thione.[56–58] indicating that the NRF2-sMAF heterodimer binds to the ARE sequence of proteasome subunit genes.[15,16,27,28,30,56–58] However, NRF2 is not involved in the comprehensive activation of proteasome subunit genes in response to proteasome inhibition.[15,16] The similarity of DNA binding motifs between NRF2 and NRF1 suggests that nuclear interacting partner proteins are critical determinants of the different target gene expression between NRF2 and NRF1.

Nuclear Binding Partner Proteins of NRF2 and NRF1

Several nuclear proteins have been identified as binding partners of NRF2 and NRF1. Initially, CREB-binding protein (CBP)/E1A-associated cellular p300 transcriptional co-activator (p300) acetyltransferases were identified as nuclear binding partners of NRF2 using a two-hybrid assay.[36] The same research group later identified the ATP-dependent chromatin remodeler Brahma Related Gene 1 (BRG1) as an interaction partner of NRF2.[40] NRF2 also interacts with a few transcription factors, including the glucocorticoid receptor (GR) and CCAAT/enhancer-binding protein beta (CEBP-beta).[59,60] These interactions create crosstalk between oxidative stress and other signaling pathways. For instance, in cells exposed to both dexamethasone, a GR agonist, and diethyl maleate (DEM), an NRF2 inducer, GR recruits histone deacetylases (HDACs) through the Neh4/5 domain of NRF2 to the promoter or enhancer regions of target genes. This leads to the suppression of the expression of NRF2 target genes.[59]

While protein complexes involving NRF1 have been identified by a research group,[61] an analysis of the "nuclear" NRF1 protein complex has not been performed. To differentiate the features of nuclear protein interaction partners between NRF2 and NRF1, we established separate 293F cell lines, each expressing NRF2 and NRF1 tagged with FLAG at the N- and C-termini, respectively. Subsequently, we identified binding partner proteins for both proteins.[41,62] This approach allowed us to conduct a comparative analysis of the binding partners of these two proteins. The protein complexes we identified in 293F cells can be categorized into three groups: 1. Common nuclear binding partners, 2. Unique nuclear binding partners of NRF2, and 3. Unique nuclear binding partners of NRF1. Each group is discussed in detail below.

CBP/p300 as an Overlapping Nuclear Binding Partner of NRF2 and NRF1

CBP and p300 are components of both NRF2 and NRF1 nuclear protein complexes (author's unpublished data).[41] CBP and p300 serve as coactivators for a broad spectrum of transcription factors, enhancing their capacity to activate gene expression.[63] These coactivators possess histone acetyltransferase (HAT) activity, specifically acetylating lysine residues on histone tails, such as histone H3K4 and K27. This acetylation modifies chromatin structure, leading to increased accessibility of tran-

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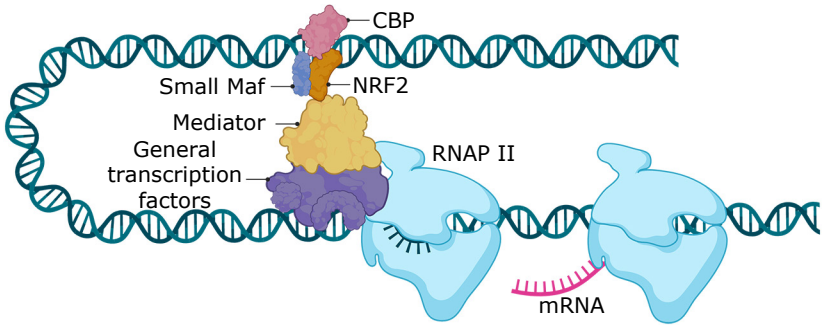

Fig. 2. Transcriptional regulatory mechanisms by NRF2 on the chromatin. NRF2 interacts with the Mediator complex via the MED16 subunit. The Mediator complex facilitates RNAP II elongation in response to oxidative stress. CBP interacts with NRF2 through its Neh4/5 domain.

scriptional activators to DNA, thus promoting gene expression. Consistent with the interaction of NRF2 with CBP/p300, our recent findings show that NRF2's chromatin binding sites correspond well with histone H3K27ac marks, typically associated with enhancer regions.[14] Several enhancers are specifically formed in NRF2-activated cancers, for instance the one in *NOTCH3* locus which contributes to tumor-initiating activity in these cancers.[14,60]

CBP and p300 also acetylate numerous transcription factors, resulting in changes to their properties, including stability and DNA binding ability. While acetylation of NRF2 regulates its DNA binding activity and nuclear localization,[64,65] no acetylated NRF1 peptides have been detected in comprehensive peptide acetylation analyses.[66,67] The functional significance of CBP and p300 as NRF1 binding partners remains to be fully elucidated.

Mediator Complex as a Unique Nuclear Binding Partner of NRF2

The Mediator complex was obtained in the nuclear NRF2 complex but not in the nuclear NRF1 complex.[41,62] The Mediator complex is an evolutionarily conserved multiprotein complex consisting of 26 subunits in mammals (21 in yeast) and plays an essential role in transcription mediated by RNA polymerase II (RNAP II).[68,69] It functions as a critical protein complex connecting DNA-binding transcription factors with the core transcription machinery, including RNAP II.

Biochemical and structural analyses have revealed the presence of four distinct modules within the Mediator complex: the head, middle, tail, and kinase modules. This diversity of subunits allows interactions with numerous transcription factors. MED1 and MED14 are subunits that interact with nuclear receptors and GATA binding protein 1 (GATA1).[70–73] Additionally, sterol regulatory element binding protein (SREBP), a transcription factor responding to lipid metabolism status, directly interacts with MED15.[74] Consequently, the Mediator complex operates as a central hub that receives and integrates various regulatory signals to trigger the induced transcription of signal-specific genes.[68] However, a specific Mediator subunit responsible for mediating oxidative stress responses remained unidentified.

Our study demonstrated that MED16 is a critical subunit for sensing oxidative stress.[41] The Mediator complex mainly interacts with NRF2 through its Neh4/5 domains, and MED16 directly binds to NRF2. *Med16* deficiency attenuates the induction of NRF2 target genes in electrophile-treated cells.[41] While the recruitment of NRF2 and CBP to the promoter of NRF2 target genes, such as *Nqo1*, *Gclm*, and *Gclc*, remains unchanged regardless of *Med16* deficiency, RNAPII S2 phosphorylation, which marks transcriptional elongation, is significantly reduced in *Med16* knockout cells.[41] This indicates that the Mediator complex is required for RNAP II-mediated transcriptional elongation, rather than RNAP II enrichment at the promoter of oxidative stress-induced genes (Fig. 2).

OGT-HCF1 as a Unique Nuclear Binding Partner of NRF1

O-linked *N*-acetylglucosamine transferase (OGT)-host cell factor C1 (HCF1) is exclusively present in the NRF1 protein complex.[62] *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) is a post-translational modification that occurs on serine and threonine residues of specific target proteins.[75] While glycosylation primarily takes place in the rough ER and the Golgi apparatus, *O*-GlcNAcylation is uniquely synthesized in the nucleus and cytoplasm. This *O*-GlcNAc modification can influence the subcellular localization, stability, and interaction specificity of target proteins.[75] The enzymatic attachment of the *O*-GlcNAc moiety is mediated by OGT.[76] OGT activation requires a substrate, uridine diphosphate *N*-acetylglucosamine (UDP-GlcNAc), which is synthesized through the hexosamine biosynthesis pathway (HBP). Availability of UDP-GlcNAc is influenced by glucose, glutamine, and glucosamine conditions, making *O*-GlcNAcylation a nutrient-sensing modification. *O*-GlcNAc modification can be dynamically controlled through its removal by *O*-GlcNAcase (OGA, also known as MGEA5).[77] Since serine and threonine, the target residues for *O*-GlcNAcylation, are also sites for phosphorylation, *O*-GlcNAcylation competes with phosphorylation at some residues.[75]

Our study demonstrated the essential role of OGT and HCF-1 in NRF1-mediated proteasome bounce-back response.[62] NRF1 undergoes *O*-GlcNAcylation at serine residues that are phosphorylated in a glycogen synthase kinase 3 beta (GSK-3β)-dependent manner, and these modifications are necessary for NRF1's interaction with β-TrCP.[33] Phosphorylation of NRF1 by GSK-3β enhances its association with β-TrCP, resulting in proteasomal degradation of NRF1. *O*-GlcNAcylation of NRF1, conversely, leads to its stabilization by antagonizing GSK-3β-mediated phosphorylation.[62]

O-GlcNAcylation is abundant in the nucleus, particularly on chromatin-binding proteins and transcription factors, underscoring its critical role as a gene expression regulator.[75] OGT is a component of several transcriptional regulator complexes, including the Polycomb complex and the tet methylcytosine dioxygenase 2 (TET2) DNA demethylase, leading to both gene suppression and activation, respectively.[78,79] Furthermore, OGT directly controls the epigenetic *O*-GlcNAc modification of Histone H2B.[80] OGT also regulates RNAP II activation through *O*-GlcNAcylation of S5 in its C-terminal domain (CTD), which inhibits transcriptional initiation, resulting in the suppression of gene transcription.[81] It remains unknown whether the interaction between NRF1 and OGT is required for chromatin-based gene expression regulation (Fig. 3).

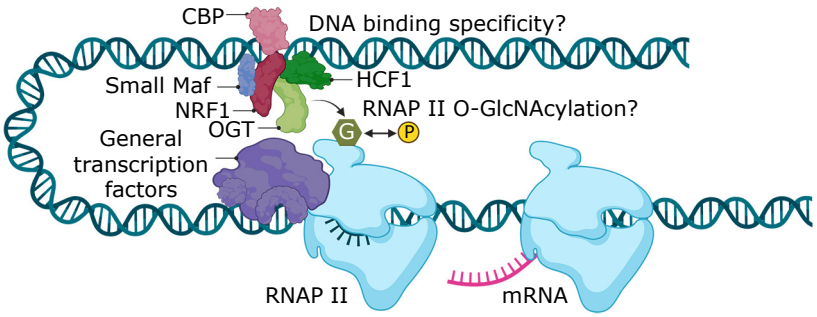

Fig. 3. Transcriptional regulatory mechanisms by NRF1 on the chromatin. NRF1 interacts with the OGT-HCF1, leading to its protein stabilization. HCF1 is a chromatin-binding factor, and OGT is involved in various transcriptional regulations. However, it remains unclear whether the OGT-HCF1 is involved in NRF1-mediated transcriptional regulation.

Concluding Remarks

While NRF2 and NRF1 share similar domain structure and exhibit DNA binding feature, they play distinct roles in the activation of their target genes.[1,30] Comparison between nuclear interacting proteins of NRF2 and those of NRF1 provides insights into understanding their differential roles in target gene activation. NRF2 is a potent transcriptional activator, possibly due to its interactions with other transcriptional activators such as the Mediator complex and CBP/p300 (Fig. 2). Notably, CBP/p300 and the Mediator complex have been observed at super-enhancers regulating gene expression in response to various stresses.[82,83] Comprehensive exploration of the chromatin localization of NRF2-the Mediator complex-CBP/p300 under the oxidative stress may reveal a new intranuclear redox response.

While the molecular mechanisms governing NRF1 activation on the ER membrane have been well-studied, the functional analysis of nuclear NRF1 as a transcriptional activator remains incomplete. Although we have demonstrated the role of OGT-HCF1 in stabilizing NRF1, further analysis of its DNA binding and transcriptional regulation on chromatin should be conducted. In contrast to NRF2, NRF1 exhibits a comparatively weaker transcriptional activity, which might be caused by OGT activity. For example, OGT recruited by NRF1 to the vicinity of RNAP II during the proteasome bounce-back response may *O*-GlcNAcylate the CTD of RNAP II, which may limit the transcriptional output of proteasome subunit genes regulated by NRF1-OGT complex. Additionally, a comprehensive assessment of colocalization between HCF1 and NRF1 will unveil DNA binding specificity of NRF1, which may distinct from that of NRF2 in the chromatin context, given the role of HCF1 as a chromatin binding factor (Fig. 3).[84,85] The final question is what structural feature of NRF1 recruits a distinct set of interacting proteins from that of NRF2.

The comparison of NRF2 and NRF1 will provide a prototypical understanding of the commonality and diversity of transcription factors that share the consensus DNA-binding motif but are involved in distinct biological processes.

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Abbreviations

ARE	antioxidant response element
BRG1	Brahma Related Gene 1
β-TrCP	beta-transducin repeat-containing protein
bZip	basic region-leucine zipper
CBP	CREB-binding protein
CEBP-beta	CCAAT/enhancer-binding protein beta
CNC	Cap'n'Collar
CUL1	Cullin 1
CUL3	Cullin 3
DDI2	DNA damage-inducible 1 homolog 2
DEM	diethyl maleate
ER	endoplasmic reticulum
GATA1	GATA binding protein 1
GR	glucocorticoid receptor
GSK-3β	glycogen synthase kinase 3 beta
HAT	histone acetyltransferase
HBP	hexosamine biosynthesis pathway
HCF1	host cell factor C1
HDACs	histone deacetylases
KEAP1	kelch-like ECH-associated protein 1
MEFs	mouse embryonic fibroblasts
NGLY1	*N*-glycanase 1
NRF1	nuclear factor erythroid 2-related factor 1
NRF2	nuclear factor erythroid 2-related factor 2
Neh	NRF2-ECH homology domain
O-GlcNAc	*O*-linked *N*-acetylglucosamine
OGA	*O*-GlcNAcase
OGT	*O*-linked *N*-acetylglucosamine transferase
p300	E1A-associated cellular p300 transcriptional co-activator
RNAP II	RNA polymerase II
ROS	reactive oxygen species
sMAF	small Maf
SREBP	sterol regulatory element binding protein
TET2	tet methylcytosine dioxygenase 2
UDP-GlcNAc	uridine diphosphate *N*-acetylglucosamine
VCP	valosin-containing protein

Conflict of Interest

No potential conflicts of interest were disclosed.

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