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Feed-Forward and Feed-Back Circuits of the NRF2/AP-1 Composite Pathway

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Abstract

Being the central regulator of oxidative status of the cell, NRF2 must be regulated so that its activity can be rapidly and strongly induced when needed and quickly suppressed when not. Moreover, for the cell, NRF2 means much more than just antioxidant defense. Numerous general functions rely on NRF2 and related factors. All this implies that the NRF2 pathway has peculiar and powerful mechanisms of control of its activity. To a great extent, these mechanisms are based on feed-forward and feed-back circuits. These circuits, more than a dozen, are in the focus of this chapter.

Keywords: feed-back regulation, feed-forward regulation, SQSTM1, SESN2, thioredoxin, NF-kappaB, GSTP1, NADPH oxidase, NRF1, NFE2L1, NRF3, NFE2L3, BACH1, 26S proteasome, MIR-144, mitochondrial biogenesis, truncated NRF2, NRF2, NFE2L2, AP-1

1. Introduction

According to the study by Malhotra et al. [1], Nrf2 controls 1055 protein-coding genes in mice. Although no similar study has ever been performed in humans and given that most routinely studied Nrf2 targets are the same in humans and mice, it is a reasonable assumption that roughly the same number of protein-coding genes is regulated by NRF2 in our species, accounting for astonishing ~5% of all our protein-coding genes. This raises a question: how does NRF2, being a well-known stand-by inducible transcription factor curbed in cytoplasm by KEAP1, fit into cellular context with so many target genes? Among those numerous genes, only a portion codes for immediate antioxidants and detoxifying enzymes. It is the more

intriguing since central proteasomal [2], autophagic [3], general signaling [4, 5], and, moreover, cell proliferation, cell cycle and survival regulation factors [1] are in fact NRF2 targets.

Although 645 of the 1055 Nrf2 target genes were found to have basal type of expression regulation by this factor [1], Nrf2 is still required to be present in the nucleus in significant amounts to drive their expression. Notably, most of these genes are not at all related to antioxidant and xenobiotic defense systems, i.e., they are essential for proliferation and basic functioning of the cell. Thus, basal expression driven by NRF2 is important for the cell, but to what extent? One should bear in mind that NRF2 knockouts are nonlethal and generally appear normal in stable laboratory conditions [6], yet they are susceptible to numerous diseases [7–9], especially when the housing conditions are suboptimal [6, 10–12]. This confirms that basal NRF2 functioning, although not vital, is critical for the cell and organism even when no pro-oxidant or xenobiotic exposure is present.

It implies that some cellular mechanisms serve to provide the cell with at least minimal necessary NRF2 activity. On the other hand, it is inherently important for an inducible antioxidant and xenobiotic defense pathway to act as quickly as possible, thus signal amplification is required whenever the initial stimulus is applied. These two distinct prerequisites for normal cell functioning in ever-changing environment appear to be resolved in principle similarly: there are feed-forward circuits that maintain steady-state level of the NRF2 activity and make signal amplification possible when fast cellular reactions are required.

Conversely, too much NRF2 activity is no better than its absence: Keap1-null mice demonstrate postnatal lethality [13, 14], an effect coinciding with nuclear Nrf2 localization [14] and reversed by Nrf2 downregulation [13]. In this case, several feed-back circuits protect the cell and organism from obsessive NRF2 activation.

Most of the feed-forward and feed-back circuits of the NRF2 pathway also involve the closely related transcription factor activator protein 1 (AP-1) represented by homodimers of Jun proteins (JUN, JUNB, JUND) or heterodimers of Fos (FOS, FOSB, FOSL1, FOSL2) and Jun proteins. There are also other dimers of Jun/Fos proteins—e.g. with Atf-proteins [15–17]—still referred to as AP-1 complexes (although either Jun or Fos proteins are absent), and oligomers of Jun/Fos proteins with other proteins. NRF2, in line with NRF1, NRF3 and AP-1 proteins (including Atf proteins) are all basic leucine zipper (bZip) transcription factors of similar structure [18–20]. Functional roles and regulation of Nrf- and AP-1-proteins also significantly overlap [21–24], and, what is even more, these two groups of proteins regulate each other at several levels, including transcription control. It should be especially noted that NRF2 binding site ARE and AP-1 binding site TRE often overlap with AP-1 being embedded into ARE [25, 26]. Having such tight relations, NRF2 and AP-1 obviously form a composite NRF2/AP-1 (or NFE2L2/AP-1) pathway, with both factors contributing much to their own functioning via shared feed-forward and feed-back circuits.

All feed-forward and feed-back circuits are represented, but not outlined or highlighted, in oxidative status interactome map (OSIM) created in our laboratory [26]. This chapter, in contrast, emphasizes on functioning of these circuits and their peculiarities.

2. Feed-forward circuits

2.1. The SESN2/SQSTM1 circuit

In 2010, Copple et al., and in 2011, Bui and Shin independently demonstrated that the autophagosomal adaptor protein SQSTM1 (also known as p62) is capable of activating the NRF2 pathway without oxidative modification of KEAP1 protein [27, 28]. This route of the NRF2 pathway activation is one of many known for today. And by the time of this important discovery, it had already been known that *SQSTM1* itself is a target gene of NRF2 [29]. Thus, SQSTM1 was found to form a feed-forward circuit capable of noncanonical activation of NRF2. The mode of action of this factor is in line with its primary function—it merely targets KEAP1 for autophagosomal degradation, and this process takes place in the cytoplasmic complex of KEAP1 and NRF2 [27, 28]. Copple et al., of immediate relevance to this chapter, also showed that *Sqstm1*-dependent activation of Nrf2 is responsible for ~50% of basal expression of classical Nrf2/AP-1 targets *Nqo1*, *Gclc* and classical Nrf2 target *Hmox1* [27].

Later on, Bae et al. [30] revealed that, in mice, *Sqstm1* actually promotes Keap1 degradation when *Sesn2* is available. A year before, Shin et al. found that *Sesn2/SESN2* is also an Nrf2/NRF2 target (**Figure 1**) [31].

Figure 1. The SESN2/SQSTM1 circuit. Dashed line marks putative interaction.

To our knowledge, to date, there are no direct evidences that the same SESN2/SQSTM1 system is required for KEAP1 degradation. However, considering evolutionary significance of autophagy and, consequently, SQSTM1, it is highly possible that this holds true for humans as it does for mice. It is also known that SESN2 works together with SQSTM1 in mitophagy, an instance of autophagy [32]. As such, we ourselves have recently tested this hypothesis in our laboratory using the RNA interference approach. We found that in basal conditions *SESN2* knockdown caused significant changes in expression of *GCLC*, *HMOX1* and *SQSTM1* (all NRF2 targets tested in the study) [33]. Indirect observations emanating from this study also suggest that actually *BACH1* expression is also affected, and BACH1 forms a peculiar feed-back circuit which will be discussed below. In the same study, in pro-oxidant exposure conditions (modeled by culturing HeLa cells in medium containing 400 μ M hydrogen peroxide for 24 h after the substance injection; 400 μ M hydrogen peroxide was found to be sublethal for HeLa cells in our previous study [34]), on the contrary, *SESN2* knockdown caused less pronounced effect on expression of the same set of genes—only

HMOX1 and *SQSTM1* had significant changes in expression [33]. To the moment of preparation of this chapter, these data are in publication progress.

All together, the existing data clearly show that SESN2/*SQSTM1* feed-forward circuit supports basal expression of NRF2/AP-1 targets and to some extent fortifies inducible reaction of this pathway.

2.2. The TXN feed-forward circuit

Thioredoxin 1, TXN, has long been known as a critical factor of activation of AP-1 transcription factor [35–37]. This TXN role has an intermediate factor—a DNA repair enzyme and a transcription factor DNA-binding promoter APEX1 [35, 36]. Interestingly, this protein is itself activated by some stimuli that are within primary focus of the NRF2/AP-1 pathway, including pro-oxidants and ionizing radiation [38], but excluding UV radiation [38], as is TXN nuclear import [39, 40].

Figure 2. The TXN feed-forward circuit. Dashed line marks putative interaction.

Years later, Iwasaki et al. found that APEX1 actually facilitates DNA binding to expression of target genes of NRF2 [23] in addition to those of AP-1, NF-kappaB and HIF1A [41]. The same was also confirmed by Shan et al. [42]. As APEX1 reduction by TXN is obligate to its function [37], TXN is a player in NRF2-driven expression, at least in some instances. In this sense, it should be mentioned that the fact that TXN forces NRF2 binding to DNA may be due to direct heterodimerization of Juns (e.g., JUND) with NRF2 [23, 43, 44], as it exactly is in case of *FTH1*, which was the gene of interest in the study by Iwasaki et al. This raises the possibility that at least in some cases TXN stimulates NRF2 binding to DNA when AP-1 partners are present. Nevertheless, TXN stimulates the NRF2/AP-1 pathway activation.

At the same time, it has already been known for a long time that TXN expression is driven by antioxidant responsive element [45, 46], and Kim et al. directly showed that thioredoxin forces its own expression [22].

TXN functioning as a transcription factor DNA-binding stimulator is conferred by its participation in disulfide-dithiol exchange reactions with APEX1. Once APEX1 has reduced itself in expense of reduced TXN, TXN has to be reduced. There are two major reductases of TXN: TXNRD1 [47] and TXNRD3 [48]. *TXNRD1* is in fact an NRF2 target itself [49]. This makes the TXN feed-forward circuit self-sufficient (**Figure 2**). We found no evidences that *TXNRD3* is an NRF2 target in human, although this is still plausible since this gene/protein is merely poorly studied.

Thioredoxin feed-forward circuit is notable in one more sense: as TRE is often embedded in ARE, one should always consider that an ARE-containing gene may in fact be regulated by AP-1 binding to embedded TRE. This is exactly the case of thioredoxin 1: in their work, Kim et al. found that Jun and Fos overexpression decreased *TXN* expression in K562 cells [22]. In our work, we have also observed negative interactions between AP-1 and NRF2 working on the same ARE (with embedded TRE) [34], and this mechanism appears to be cell-context dependent, since NRF2 and AP-1 can have both positive and negative effects on the same genes in different cells. This will be discussed in detail in the next section.

Interestingly, TXN feed-forward circuit appears to involve a mechanism of adjustment, which is based on differential control of *TXN* transcripts. Among NRF2 targets, *BACH1* was the first to be described as having individual control of transcripts [50]. Later, we tested whether *TXN* transcript variants are differentially expressed when the NRF2 pathway gets activated, and so it was [51, 52]. This renders the TXN feed-forward circuit easily adjustable by the cell since only one of the transcripts is NRF2/AP-1 dependent.

The existence of this feed-forward circuit was proven experimentally. However, there are at least two more circuits in the NRF2/AP-1 pathway involving TXN, NRF2 and AP-1. One of them is a mixed feed-forward/feed-back circuit depending on cellular context (discussed in next section), whereas another is a feed-back circuit (discussed below with other feed-back circuits).

2.3. The AP-1/TXN/NRF2 bidirectional circuit

As *TXN* is an NRF2 target, and TXN is required for AP-1 activation, it forms one more complex circuit. This circuit cannot be considered as either purely feed forward or feed-back. In a set of studies, it was shown that JUN, being a part of AP-1, controls expression of *NRF2* [34, 53, 54]. The complexity is that JUN can both activate and suppress the *NRF2* expression depending on the cells analyzed: DeNicola et al. [53] demonstrated that, in murine MEF K-RasG12D cells, Jun transactivates *Nrf2*, whereas Cho et al. [54] and our laboratory [34] showed that JUN has negative influence on *NRF2* expression.

Thus, in the cells that are featured by JUN activating *NRF2*, JUN stimulates expression of *NRF2*, the latter activates expression of *TXN*, which, in turn, induces JUN DNA binding. In this case, this circuit acts as feed-forward for both NRF2 and AP-1 (**Figure 3A**).

In contrast, in cells whose JUN suppresses *NRF2*, less TXN is expressed and that is less favorable for AP-1 DNA binding, thus releasing suppression from NRF2 expression. Consequently, in this case, the circuit acts as feed-back for AP-1, whereas feed-forward, to some

extent, for NRF2 (**Figure 3B**). It appears that this system favors NRF2 functioning in basal conditions, but acts to suppress it once NRF2 is activated. We ourselves observed the latter situation [34] but have not yet tested the former.

Figure 3. The AP-1/TXN/NRF2 bidirectional circuit. Dashed line marks putative interaction. (A) depicts the situation when cell signaling background determines the system of interaction between NRF2 and AP-1 to act as a feed-forward circuit. On the other hand, (B) illustrates how these interactions form a feed-back circuit in other cell types or cellular conditions.

How is this possible that this circuit differs this much in various cells? The simplest explanation we would suggest is, different cell lines express different amounts of Fos proteins—FOS, FOSB, FOSL1, FOSL2, as well as other Jun proteins—JUNB, JUND [22, 35, 55]. All these proteins significantly differ in their effects on JUN. Moreover, how all Juns perform depends on the presence of JUN dimerizing protein JDP2 capable of deactivating Juns by dimerizing them and by epigenetically silencing the *JUN* gene [56]. Thus, immediate protein partners of JUN and its powerful regulator JDP2 seem to determine whether JUN will activate or suppress the NRF2 expression in a given cell line. However, this is to be tested in upcoming studies.

2.4. The GSTP1 feed-forward circuit

One of the well-known NRF2 targets, *GSTP1* [57, 58], has recently been found to form a novel feed-forward circuit of the NRF2/AP-1 pathway. Carvalho et al. have just recently revealed that, in mice, *Gstp1* is capable of S-glutathionylation of Keap1 disrupting the interactions between Keap1 and Nrf2 (**Figure 4**) [59].

Although this has not been observed in humans to date, it is plausible that the same circuit is also characteristic of the human cell. It is also tempting to suggest that other glutathione-S-transferases may have the same function. At least *GSTA1* [60] and *GSTA4* [61] expression is, meanwhile, subject to NRF2 regulation.

Figure 4. The GSTP1 feed-forward circuit. GS-S-Keap1—S-glutathionylated Keap1.

Of importance, *GSTP1* is activated by both AP-1 and NRF2. Thus, whenever AP-1 is activated, NRF2 is released of KEAP1. This may be a mechanism of protective coupling of cellular events because AP-1 has much more profound effects on the cellular physiology than NRF2 does.

2.5. The NOX4 feed-forward circuit

Among peculiar NRF2 targets is NOX4 [62, 63]. This protein is a part of one of NADPH oxidase multiprotein complexes. The inherent NOX4 function is production of superoxide anion, similarly to other Noxes. Two features of this enzyme with respect to other enzymes of the family are that (1) NOX4 is strongly expressed in kidney where it is suggested to serve as an oxygen sensor for controlling the erythropoietin production [64, 65], and (2) NOX4 is localized to endoplasmic reticulum [66] and nucleus [65] in addition to cell membrane.

Barring NOX5 [67], Noxes require partners for functioning. In case of NOX4, the obligate partner is only one—the CYBA protein (widely known as p22phox) [68]. Thus, the NOX4 feed-forward circuit would require CYBA for functioning, and CYBA is not an NRF2 target.

However, CYBA is in fact an NF-kappaB and AP-1 target [69]. Thus, the composite NRF2/AP-1 pathway controls both enzymes necessary for the NOX4 feed-forward circuit (**Figure 5**).

Figure 5. The NOX4 feed-forward circuit.

Additionally, NF-kappaB is activated by pro-oxidants [40, 70–72], and DNA binding of both AP-1 and NF-kappaB is stimulated by TXN [40, 70–73].

3. Feed-back circuits

As seen from data above, the cellular feed-forward circuits of the NRF2/AP-1 pathway are numerous and versatile providing the cell with required steady-state levels of the proteins, and signal amplification upon induction of the pathway. However, as already mentioned, hyperactive NRF2 and AP-1 are a death threat to the organism. If NRF2 causes disturbances of tissues functioning, AP-1 is a pro-oncogenic transcription factor (JUN and FOS proteins are also, and in some areas—ordinarily, known as c-jun and c-fos proto-oncogenes) [74, 75]. In addition to that, NRF2 controls autophagy through SQSTM1, and also transactivates several ABC-transporters, including *ABCB1* (*MDR1*), *ABCG2* (*BCRP*), *ABCC2* (*MRP2*) and *ABCC3* (*MRP3*) [76]—these are all major factors of carcinogenesis and cancer progression. Please note that although autophagy suppresses malignization, once tumor turns malignant, autophagy becomes a cancer-promoting metabolic and drug resistance-conferring trait [77].

Thus, NRF2 and AP-1 activity should be tightly controlled once activated. Several feed-back circuits allow for that. Two of them involve factors participating in feed-forward circuits. These circuits are the first to be discussed.

3.1. The TXN feed-back circuit

TXN localizes to cytoplasm and migrates to nucleus upon stimulation. However, the cytoplasmic pool of thioredoxin 1 provides the cell with antioxidant capacity. Thus, cytoplasmic TXN inhibits pro-oxidants-induced NF-kappaB and NRF2 nuclear import [72].

Consequently, as soon as the NRF2/AP-1 pathway activation produces substantial amounts of thioredoxin 1, TXN stabilizes KEAP1 and promotes NRF2 sequestration and degradation (Figure 6). This works wherever KEAP1 and TXN appear, as will be discussed later.

Figure 6. The TXN feed-back circuit. Dotted line marks an indirect effect.

3.2. The NRF3/NOX4 feed-back circuit

One of the least studied, if not the worst studied, bZip proteins is the third homolog of NRF2 —NRF3. Very little is known about this transcription factor except the fact that it usually antagonizes NRF1 and NRF2 [22, 78, 79]. Even less is known on stimuli that activate NRF3. NRF3 knockout is known not to change the phenotype to any notable extent [80].

However, Zhang et al. and Pepe et al. revealed that NRF3 is activated by ER stress inducers [81, 82]. Once activated, NRF3, predictably, suppresses ordinary NRF2 target genes [82]. Surprisingly, in contrast to these genes, *NOX4* was activated by NRF3 [82]. As NOX4 is an enzyme localized to endoplasmic reticulum and producing superoxide anion, it is capable of oxidative modification of inositol 1,4,5-trisphosphate receptors [83]. Once oxidized, these receptors cause calcium efflux from endoplasmic reticulum to cytosol. This event leads to endoplasmic reticulum stress [83].

As mentioned above, NOX4, for its functioning, requires an AP-1 target—CYBA. ER stress is not a leading, if at all, stimulus for AP-1 activation, yet ER stress causes ROS production (during the protein refolding period), and ROS activation is an acknowledged property of AP-1 [35]. Additionally, NRF3 heterodimerizes with AP-1 proteins FOS and FOSL1 (it was suggested by Zhang et al. that NRF3 thus suppresses TRE-containing NRF2 targets only [81]) and is suggested to heterodimerize with Jun proteins (**Figure 7**) [79, 84].

Figure 7. The NRF3/NOX4 feed-back circuit. Dashed line marks putative interaction.

The fact that NRF3 activates *NOX4* also suggests that the NOX4 feed-forward circuit may equally work for both NRF2 and AP-1.

3.3. The KEAP1 feed-back circuit

KEAP1 is the immediate antagonist of NFR2 as it sequesters it and targets it for proteasomal degradation. In a complex experiment, Lee et al. demonstrated that murine *Keap1* is another Nrf2 target [85]. Unfortunately, no direct evidences that the same is true for humans are known to the authors to date of preparation of this chapter. This is an extremely important issue to be addressed in future studies. Nevertheless, at least in mice, this circuit is active and facilitates fast Nrf2 pathway shutdown as soon as the oxidative status of the cell is normalized (**Figure 8**).

Interestingly, KEAP1 can be imported into the nucleus by KPNA6 [46, 60, 86]. In the nucleus, KEAP1 acts exactly as in the cytoplasm—it binds NFE2L2 and induces its polyubiquitination thus targeting it for degradation [46, 85].

It means that GSK3B activity providing NRF2 nuclear export [87–89] is not even required for KEAP1 to force NRF2 degradation. However, KEAP1 nuclear import is a tightly controlled process. An example of the chain of events causing KEAP1 nuclear import is presented in the next section.

Figure 8. The KEAP1 feed-back circuit.

3.4. The NF-kappaB/KEAP1 feed-back circuit

KEAP1 nuclear import stimulation is well described for the case of NF-kappaB activation. Generally, the NF-kappaB and NRF2 pathways antagonize in several ways: NF-kappaB protein RELA (p65) promotes HDAC3 interaction with CREBBP or MAFK thus causing local hypoacetylation surrounding ARE [90]; KEAP1 promotes IKK degradation thus suppressing NF-kappaB release from IKBs [91]; and, in turn, RELA promotes KEAP1 nuclear import [46].

Thus, whenever NF-kappaB is activated, it suppresses NFE2L2 by two mechanisms: by inducing its nuclear sequestration by KEAP1 and by causing transcription-suppressing epigenomic modification of the NRF2-dependent loci. In this sense, the NF-kappaB/KEAP1 circuit presented here is feed-back for the NRF2/AP-1 pathway yet feed-forward for the NF-kappaB pathway, because less KEAP1 causes less IKK degradation consequently promoting NF-kappaB nuclear import. This feed-forward circuit is probably disrupted by TXN, which is NRF2-dependent (Figure 9).

Figure 9. The NF-kappaB/KEAP1 feed-back circuit.

3.5. The BACH1 feed-back circuit

In contrast to the preferentially cytoplasmic NRF2 inhibitor KEAP1, BACH1 is its nuclear antagonist. Just as NRF2, BACH1 belongs to the bZip family, and moreover, to cap'n'collar

(CNC) sub-family. Thus, BACH1 and NRF2 act in a very similar manner—by heterodimerizing with small Maf proteins [86, 92–94]. There is, however, one striking difference between the two transcription factors: BACH1 acts only on clustered-ARE genes [49, 93], like *HMOX1* [93, 95] and *NQO1* [96].

As it was discussed earlier, different NRF2 targets may respond distinctly to the pathway stimulation or release of pathway suppression. This actually may be caused by BACH1. For example, *TXNRD1* is proven to be BACH1-independent [49], while *HMOX1* has quadruple ARE [93] being subject to BACH1-induced suppression, and *NQO1* has a double ARE still being somewhat suppressed by BACH1 [96]. It appears that BACH1 functioning allows the cell to discriminate between the NRF2 targets. It is achieved by a relatively simple mechanism: if NRF2 activity is induced by whatever mechanism or stimulus except for pro-oxidant exposure, BACH1 does not allow NRF2 to act on the clustered-ARE genes. In contrast, when the cell is exposed to pro-oxidants of whatever nature, BACH1 is fast oxidized [19, 49, 86, 93, 96, 97], rapidly detaches from DNA [93, 94, 98] and readily degrades [93, 97].

At the same time, *BACH1* is itself an NRF2 target and an extremely interesting one. The *BACH1* locus codes for three transcript variants produced as the result of alternative transcription initiation. Only *BACH1* transcript variant 2 is NRF2-dependent: *BACH1* intronic +1411 nt ARE is functional and promotes transcription of the second intron of the gene [50]. The authors of this discovery, Jyrkkänen et al., proposed the existence of the BACH1 feed-back circuit along with their discovery of the functional intronic ARE [50].

The existence of the BACH1 feed-back circuit predisposes different expression dynamics of NRF2 targets depending on their ARE structure: once the NRF2 pathway normalizes oxidative status of the cell, newly synthesized BACH1 protein successfully outcompetes NRF2 on the clustered-ARE loci, while allowing it to act further on non-clustered ARE genes (**Figure 10**).

Figure 10. The BACH1 feed-back circuit.

In one of our studies, we appeared to observe these events after we released the JUN-imposed suppression of NRF2 in cells treated with hydrogen peroxide: in contrast non-clustered ARE genes (*FTH1*, *CBR3*, *SQSTM1*), *HMOX1* expression did not rise—probably because BACH1, being a non-clustered ARE gene, had increased expression in these those settings [34].

3.6. The 26S proteasome/NRF2 feed-back circuit

Once polyubiquitinated, NRF2 is degraded by 26S proteasome [86]. Proteasome inhibitors suppress the degradation of NRF2 and stimulate its nuclear import [99].

At the same time, as shown in mice, Nrf2 controls at least eight genes of proteasomal proteins: *Psm1*, *Psm4*, *Psm3*, *Psm5*, *Psm6*, *Psm1*, *Psm3* and *Psm14* (**Figure 11**) [2].

Figure 11. The 26S proteasome/NRF2 feed-back circuit.

To date of preparation of the chapter, no similar results obtained from studies on human cells have been published. Nevertheless, considering the biological significance of the 26S proteasome, it is not an unfair assumption that the same genes are probably controlled by NRF2 in humans.

Notably, even in human cells, AP-1 controls expression of interferon gamma [100], which, in turn, transactivates *PSME1* [101] and *PSME2* [102] subunit genes of the 26S proteasome.

Thus, NRF2 pathway activation facilitates NRF2 proteasomal degradation once KEAP1 is re-enabled to sequester NRF2. At least, this system works in mice, and probably in humans, too, especially since two *Psm* genes are under the NRF2/AP-1 pathway control.

3.7. The MIR-144 feed-back circuit

One of numerous NRF2-antagonizing miRNAs, *MIR-144*, is expressed under control of AP-1 [103]. This miRNA not merely blocks translation of NRF2, but rather degrades the *NRF2* mRNA [104, 105].

3.8. The mitochondrial bidirectional circuit

Being regulated by reactive oxygen species and signaling background of the cell, the NRF2/AP-1 pathway depends on mitochondrial function, including their vast signaling activity [106]. Surprisingly, the NRF2/AP-1 pathway has been found to modulate mitochondrial biogenesis by two mechanisms. The first one has direct experimental evidences: NRF2 is a transcriptional regulator of *NRF1*—nuclear respiratory factor (which is not to be confused with *NFE2L1*, an

NRF2 homolog, also known and referred to as *NRF1* in this chapter) [107]. *NRF1*, in turn, controls a battery of nuclear genes required for mitochondrial biogenesis and function [107]. The second mechanism has not been observed in direct experiments and is related to the fact that the *NRF1* protein is also controlled by estrogen receptors—a distinct family of transcription factors [108]. However, it is well-known that estrogen receptors act in an intimate collaboration with AP-1 proteins [109, 110]. For example, FOS alone is required for expression of 37% of estrogen receptors target genes [111]. Even though the mitochondrial biogenesis-related targets of the estrogen receptors may not fall into the AP-1-dependent category, which is to be addressed in the future, there is another doubtless interaction between the estrogen receptors and the NRF2/AP-1 pathway. The already discussed NRF2/AP-1 target, *TXNRD1* is a strong promoter of transactivatory function of the estrogen receptors [111], thus the NRF2/AP-1 pathway positively regulates estrogen receptors function, while estrogen receptors facilitate mitochondria biogenesis.

Although mitochondrial biogenesis eventually leads to increased ROS generation, this circuit is unlikely to be purely or even mostly feed-forward. Rather, this circuit is bidirectional with strong feed-back action because the outer mitochondrial membrane carries the PGAM5 protein. PGAM5, in turn, tethers the NRF2-KEAP1 complexes to mitochondria and acts as a powerful suppressor of the NRF2-dependent expression (**Figure 12**) [106, 112].

Figure 12. The mitochondrial bi-directional circuit. NRF1 here is nuclear respiratory factor 1.

Thus, the more mitochondria, the more KEAP1-NRF2 complexes are likely to be bound to them, leading to decreased availability of NRF2 to nucleus. Unfortunately, it is not known to date how PGAM5 expression is controlled with relation to mitochondria biogenesis—the protein is poorly studied.

It is worth noting that the PGAM5/KEAP1-NRF2 system is suggested to represent a stand-by alarm inducible by changes in mitochondrial function [106, 112]. PGAM5 also appears to stimulate mitophagy as a part of cellular defense against excessive reactive oxygen species [113]. It could be suggested that this function of PGAM5 is directly related to its sequestering of NRF2 with subsequent release of it upon mitochondrial dysfunction: NRF2 controls expression of *SQSTM1* and *SESN2*, two factors of autophagy/mitophagy already discussed above [32, 114].

3.9. The NRF2 bidirectional circuit

NRF2 expression is known to respond to stimuli that activate the NRF2/AP-1 pathway [34, 51, 115]. We and others observed that, for reasons still to be revealed, *NRF2* had a humped expression curve when different cells had been treated with various pro-oxidant substances [34, 115]. It is possible that this is related to the AP-1/TXN/NRF2 bidirectional circuit. It is tempting to suggest that this is caused by AP-1 components that are expressed with different dynamics upon stimulation.

For example, Siriani et al. demonstrated, that angiotensin II, over the same period of treatment, induced 300-fold change in expression of *FOS*, 500-fold change in expression of *FOSB*, 2-fold change in expression of *FOSL1* and *FOSL2*, 4-fold change in expression of *JUN*, 14-fold change in expression of *JUNB* and 2-fold change in expression of *JUND*. The same was also fair for the classical AP-1 inducer—TPA: it caused 194-fold change in expression of *FOS*, 65-fold change in expression of *FOSB*, 2-fold change in expression of *FOSL1*, 2-fold change in expression of *JUN* and *JUND*, and 9-fold change in expression of *JUNB* [116].

Since overflow inhibition is characteristic of AP-1 [117], and competition of bZip dimers is a must, it is possible that alterations in ratio of AP-1 components controlling *NRF2* expression lead to its humped dosage-expression curve.

This circuit cannot be considered purely feed-back, because before certain threshold, the stimulus activates *NRF2* expression. Yet, this character of expression of *NRF2* is not entirely of feed-forward nature, and this is discussed in the next, last, section of this chapter.

3.10. The truncated NRF2 feed-back circuit

NRF2 is cleaved into a truncated form named truncated *NRF2*, or t*NRF2*, by Ice family-like caspases [118]. Just as the full-length protein, t*NRF2* enters nucleus, but there it antagonizes the normal *NRF2* and suppresses ARE-containing loci [19, 118].

4. Conclusion

Although studying the NRF2/AP-1 pathway targets is an extremely complex task due to their plentitude, experimental evidences suggest that this relatively small, with respect to the regulatory proteins, pathway has enormous number of feed-forward and feed-back circuits, some of them being bidirectional.

Some evidences demonstrated directly that these circuits contribute to either fast activation or quenching of the pathway. For other circuits, no such testing has ever been performed. Nevertheless, the existing data unambiguously point out that these circuits are an essential feature of the NRF2/AP-1 pathway. These circuits must be accounted in all applications—starting from cell sensor-based pharmacological screening techniques [119, 120] and ending with therapeutical research and development [121].

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References

- [1] Malhotra D, Portales-Casamar E, Singh A, Srivastava S, Arenillas D, Happel C, Shyr C, Wakabayashi N, Kensler TW, Wasserman WW, Biswal S. Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acids Res.* 2010;38(17):5718–5734. doi:10.1093/nar/gkq212.
- [2] Kwak MK, Wakabayashi N, Greenlaw JL, Yamamoto M, Kensler TW. Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. *Mol Cell Biol.* 2003;23(23):8786–8794.
- [3] Taguchi K, Fujikawa N, Komatsu M, Ishii T, Unno M, Akaike T, Motohashi H, Yamamoto M. Keap1 degradation by autophagy for the maintenance of redox homeostasis. *Proc Natl Acad Sci USA.* 2012;109(34):13561–13566. doi:10.1073/pnas.1121572109.
- [4] Erttmann SF, Bast A, Seidel J, Breitbach K, Walther R, Steinmetz I. PGD2 and PGE2 regulate gene expression of Prx 6 in primary macrophages via Nrf2. *Free Radic Biol Med.* 2011;51(3):626–640. doi:10.1016/j.freeradbiomed.2011.05.022.
- [5] Ho JN, Lee SB, Lee SS, Yoon SH, Kang GY, Hwang SG, Um HD. Phospholipase A2 activity of peroxiredoxin 6 promotes invasion and metastasis of lung cancer cells. *Mol Cancer Ther.* 2010;9(4):825–832. doi:10.1158/1535-7163.MCT-09-0904.
- [6] Priestley JR, Kautenburg KE, Casati MC, Endres BT, Geurts AM, Lombard JH. The NRF2 knockout rat: a new animal model to study endothelial dysfunction, oxidant stress, and microvascular rarefaction. *Am J Physiol Heart Circ Physiol.* 2016;310(4):H478–487. doi:10.1152/ajpheart.00586.2015.
- [7] Khor TO, Huang MT, Prawan A, Liu Y, Hao X, Yu S, Cheung WK, Chan JY, Reddy BS, Yang CS, Kong AN. Increased susceptibility of Nrf2 knockout mice to colitis-associated

- colorectal cancer. *Cancer Prev Res (Phila)*. 2008;1(3):187–191. doi:10.1158/1940-6207.CAPR-08-0028.
- [8] Beyer TA, Xu W, Teupser D, auf dem Keller U, Bugnon P, Hildt E, Thiery J, Kan YW, Werner S. Impaired liver regeneration in Nrf2 knockout mice: role of ROS-mediated insulin/IGF-1 resistance. *EMBO J*. 2008;27(1):212–223.
- [9] Cheung KL, Lee JH, Khor TO, Wu TY, Li GX, Chan J, Yang CS, Kong AN. Nrf2 knockout enhances intestinal tumorigenesis in Apc(min/+) mice due to attenuation of anti-oxidative stress pathway while potentiates inflammation. *Mol Carcinog*. 2014;53(1):77–84. doi:10.1002/mc.21950.
- [10] Furnari MA, Saw CL, Kong AN, Wagner GC. Altered behavioral development in Nrf2 knockout mice following early postnatal exposure to valproic acid. *Brain Res Bull*. 2014;109:132–142. doi:10.1016/j.brainresbull.2014.10.006.
- [11] Chartoumpekis DV, Ziros PG, Zaravinos A, Iskrenova RP, Psyrogiannis AI, Kyriazopoulou VE, Sykiotis GP, Habeos IG. Hepatic gene expression profiling in Nrf2 knockout mice after long-term high-fat diet-induced obesity. *Oxid Med Cell Longev*. 2013;2013:340731. doi:10.1155/2013/340731.
- [12] Becks L, Prince M, Burson H, Christophe C, Broadway M, Itoh K, Yamamoto M, Mathis M, Orchard E, Shi R et al. Aggressive mammary carcinoma progression in Nrf2 knockout mice treated with 7,12-dimethylbenz[a]anthracene. *BMC Cancer*. 2010;10:540. doi:10.1186/1471-2407-10-540.
- [13] Wakabayashi N, Itoh K, Wakabayashi J, Motohashi H, Noda S, Takahashi S, Imakado S, Kotsuji T, Otsuka F, Roop DR et al. Keap1-null mutation leads to postnatal lethality due to constitutive Nrf2 activation. *Nat Genet*. 2003;35(3):238–245.
- [14] Satoh T, Harada N, Hosoya T, Tohyama K, Yamamoto M, Itoh K. Keap1/Nrf2 system regulates neuronal survival as revealed through study of keap1 gene-knockout mice. *Biochem Biophys Res Commun*. 2009;380(2):298–302. doi:10.1016/j.bbrc.2009.01.063.
- [15] Juilland M, Gonzalez M, Erdmann T, Banz Y, Jevnikar Z, Hailfinger S, Tzankov A, Grau M, Lenz G, Novak U et al. CARMA1- and MyD88-dependent activation of Jun/ATF-type AP-1 complexes is a hallmark of ABC diffuse large B-cell lymphomas. *Blood*. 2016;127(14):1780–1789. doi:10.1182/blood-2015-07-655647.
- [16] Gozdecka M, Breitwieser W. The roles of ATF2 (activating transcription factor 2) in tumorigenesis. *Biochem Soc Trans*. 2012;40(1):230–234. doi:10.1042/BST20110630.
- [17] Srivastava PK, Hull RP, Behmoaras J, Petretto E, Aitman TJ. JunD/AP1 regulatory network analysis during macrophage activation in a rat model of crescentic glomerulonephritis. *BMC Syst Biol*. 2013;7:93. doi:10.1186/1752-0509-7-93.
- [18] Novotny V, Prieschl EE, Csonga R, Fabjani G, Baumruker T. Nrf1 in a complex with fosB, c-jun, junD and ATF2 forms the AP1 component at the TNF alpha promoter in stimulated mast cells. *Nucleic Acids Res*. 1998;26(23):5480–5485.

- [19] Sykietis GP, Bohmann D. Stress-activated cap'n'collar transcription factors in aging and human disease. *Sci Signal*. 2010;3(112):re3. doi:10.1126/scisignal.3112re3.
- [20] Babu RL, Naveen Kumar M, Patil RH, Devaraju KS, Ramesh GT, Sharma SC. Effect of estrogen and tamoxifen on the expression pattern of AP-1 factors in MCF-7 cells: role of c-Jun, c-Fos, and Fra-1 in cell cycle regulation. *Mol Cell Biochem*. 2013;380(1–2):143–151. doi:10.1007/s11010-013-1667-x.
- [21] Venugopal R, Jaiswal AK. Nrf2 and Nrf1 in association with Jun proteins regulate antioxidant response element-mediated expression and coordinated induction of genes encoding detoxifying enzymes. *Oncogene*. 1998;17(24):3145–3156.
- [22] Kim YC, Yamaguchi Y, Kondo N, Masutani H, Yodoi J. Thioredoxin-dependent redox regulation of the antioxidant responsive element (ARE) in electrophile response. *Oncogene*. 2003;22(12):1860–1865.
- [23] Iwasaki K, Mackenzie EL, Hailemariam K, Sakamoto K, Tsuji Y. Hemin-mediated regulation of an antioxidant-responsive element of the human ferritin H gene and role of Ref-1 during erythroid differentiation of K562 cells. *Mol Cell Biol*. 2006;26(7):2845–2856.
- [24] Xanthoudakis S, Miao G, Wang F, Pan YC, Curran T. Redox activation of Fos-Jun DNA binding activity is mediated by a DNA repair enzyme. *EMBO J*. 1992;11(9):3323–3335.
- [25] Reichard JF, Petersen DR. Hepatic stellate cells lack AP-1 responsiveness to electrophiles and phorbol 12-myristate-13-acetate. *Biochem Biophys Res Commun*. 2004;322(3):842–853. doi:10.1016/j.bbrc.2004.07.180
- [26] Zolotukhin P, Kozlova Y, Dovzhik A, Kovalenko K, Kutsyn K, Aleksandrova A, Shkurat T. Oxidative status interactome map: towards novel approaches in experiment planning, data analysis, diagnostics and therapy. *Mol Biosyst*. 2013;9(8):2085–2096. doi:10.1039/c3mb70096h.
- [27] Copple IM, Lister A, Obeng AD, Kitteringham NR, Jenkins RE, Layfield R, Foster BJ, Goldring CE, Park BK. Physical and functional interaction of sequestosome 1 with Keap1 regulates the Keap1-Nrf2 cell defense pathway. *J Biol Chem*. 2010;285(22):16782–16788. doi:10.1074/jbc.M109.096545.
- [28] Bui CB, Shin J. Persistent expression of Nqo1 by p62-mediated Nrf2 activation facilitates p53-dependent mitotic catastrophe. *Biochem Biophys Res Commun*. 2011;412(2):347–352. doi:10.1016/j.bbrc.2011.07.101.
- [29] Jain A, Lamark T, Sjøttem E, Larsen KB, Awuh JA, Øvervatn A, McMahon M, Hayes JD, Johansen T. p62/SQSTM1 is a target gene for transcription factor NRF2 and creates a positive feedback loop by inducing antioxidant response element-driven gene transcription. *J Biol Chem*. 2010;285(29):22576–22591. doi:10.1074/jbc.M110.118976.
- [30] Bae SH, Sung SH, Oh SY, Lim JM, Lee SK, Park YN, Lee HE, Kang D, Rhee SG. Sestrins activate Nrf2 by promoting p62-dependent autophagic degradation of Keap1 and

- prevent oxidative liver damage. *Cell Metab.* 2013;17(1):73–84. doi:10.1016/j.cmet.2012.12.002
- [31] Shin BY, Jin SH, Cho IJ, Ki SH. Nrf2-ARE pathway regulates induction of Sestrin-2 expression. *Free Radic Biol Med.* 2012;53(4):834–841. doi:10.1016/j.freeradbiomed.2012.06.026.
- [32] Kim MJ, Bae SH, Ryu JC et al. SESN2/sestrin2 suppresses sepsis by inducing mitophagy and inhibiting NLRP3 activation in macrophages. *Autophagy.* 2016;12(8):1272–1291. doi: 10.1080/15548627.2016.1183081.
- [33] Belanova AA, Smirnov DS, Makarenko MS, Belousova MM, Zolotukhin PV. SESN2 knockdown deregulates expression of NFE2L2-dependent genes in HeLa cells in basal conditions and under pro-oxidant exposure. In press.
- [34] Belanova AA, Smirnov DS, Makarenko MS, Kornienko IV, Kurbatov SV, Belousova MM, Aleksandrova AA, Kharchenko EY, Prazdnova EV, Zolotukhin PV. JUN knock-down reinforces expression of NFE2L2-dependent genes in hydrogen peroxide-treated HeLa cells. *Indian J Exp Biol.* 2015; publication in process, revision 1.
- [35] Sun Y, Oberley LW. Redox regulation of transcriptional activators. *Free Radic Biol Med.* 1996;21(3):335–348.
- [36] Abate C, Patel L, Rauscher FJ 3rd, Curran T. Redox regulation of fos and jun DNA-binding activity in vitro. *Science.* 1990;249(4973):1157–1161.
- [37] Brandes N, Schmitt S, Jakob U. Thiol-based redox switches in eukaryotic proteins. *Antioxid Redox Signal.* 2009;11(5):997–1014. doi:10.1089/ARS.2008.2285.
- [38] Ramana CV, Boldogh I, Izumi T, Mitra S. Activation of apurinic/apyrimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. *Proc Natl Acad Sci USA.* 1998;95(9):5061–5066.
- [39] Wei SJ, Botero A, Hirota K, Bradbury CM, Markovina S, Laszlo A, Spitz DR, Goswami PC, Yodoi J, Gius D. Thioredoxin nuclear translocation and interaction with redox factor-1 activates the activator protein-1 transcription factor in response to ionizing radiation. *Cancer Res.* 2000;60(23):6688–6695.
- [40] Hirota K, Murata M, Sachi Y, Nakamura H, Takeuchi J, Mori K, Yodoi J. Distinct roles of thioredoxin in the cytoplasm and in the nucleus. A two-step mechanism of redox regulation of transcription factor NF-kappaB. *J Biol Chem.* 1999;274(39):27891–27897.
- [41] Li Y, Liu X, Zhou T, Kelley MR, Edwards P, Gao H, Qiao X. Inhibition of APE1/Ref-1 redox activity rescues human retinal pigment epithelial cells from oxidative stress and reduces choroidal neovascularization. *Redox Biol.* 2014;2:485–494. doi:10.1016/j.redox.2014.01.023.
- [42] Shan JL, He HT, Li MX, Zhu JW, Cheng Y, Hu N, Wang G, Wang D, Yang XQ et al. APE1 promotes antioxidant capacity by regulating Nrf-2 function through a redox-depend-

- ent mechanism. *Free Radic Biol Med*. 2015;78:11–22. doi:10.1016/j.freeradbiomed.2014.10.007.
- [43] Tsuji Y. JunD activates transcription of the human ferritin H gene through an antioxidant response element during oxidative stress. *Oncogene*. 2005;24(51):7567–7578.
- [44] Iwasaki K, Hailemariam K, Tsuji Y. PIAS3 interacts with ATF1 and regulates the human ferritin H gene through an antioxidant-responsive element. *J Biol Chem*. 2007;282(31):22335–22343.
- [45] Kim YC, Masutani H, Yamaguchi Y, Itoh K, Yamamoto M, Yodoi J. Hemin-induced activation of the thioredoxin gene by Nrf2. A differential regulation of the antioxidant responsive element by a switch of its binding factors. *J Biol Chem*. 2001;276(21):18399–18406.
- [46] Yu M, Li H, Liu Q, Liu F, Tang L, Li C, Yuan Y, Zhan Y, Xu W et al. Nuclear factor p65 interacts with Keap1 to repress the Nrf2-ARE pathway. *Cell Signal*. 2011;23(5):883–892. doi:10.1016/j.cellsig.2011.01.014.
- [47] Gasdaska PY, Gasdaska JR, Cochran S, Powis G. Cloning and sequencing of a human thioredoxin reductase. *FEBS Lett*. 1995;373(1):5–9.
- [48] Matsuzaka Y, Okamoto K, Mabuchi T, Iizuka M, Ozawa A, Oka A, Tamiya G, Kulski JK, Inoko H. Identification and characterization of novel variants of the thioredoxin reductase 3 new transcript 1 TXNRD3NT1. *Mamm Genome*. 2005;16(1):41–49.
- [49] Reichard JF, Motz GT, Puga A. Heme oxygenase-1 induction by NRF2 requires inactivation of the transcriptional repressor BACH1. *Nucleic Acids Res*. 2007;35(21):7074–7086.
- [50] Jyrkkänen HK, Kuosmanen S, Heinäniemi M, Laitinen H, Kansanen E, Mella-Aho E, Leinonen H, Ylä-Herttuala S, Levonen AL. Novel insights into the regulation of antioxidant-response-element-mediated gene expression by electrophiles: induction of the transcriptional repressor BACH1 by Nrf2. *Biochem J*. 2011;440(2):167–174. doi:10.1042/BJ20110526.
- [51] Zolotukhin PV, Dovzhik AD, Lebedeva UA, Kuzminova ON, Mashkina EV, Aleksandrova AA, Shkurat TP. Testing the concept of the interatomic status of the NFE2L2/AP1 pathway as a systemic biomarker for examination stress. *Mol Diagn Ther*. 2014;18(3):355–369. doi:10.1007/s40291-014-0088-1.
- [52] Dovzhik AD, Zolotukhin PV, Mayboroda EA, Mashkina EV, Aleksandrova AA. Assessing the regulation dependencies of thioredoxin 1 transcript variants by means of interactomic dynamic-with-induction profiling approach. *Living and life-remodelled systems*. [Zhiviye i biokostniye sistemy]. 2014;2014(6). ID 6–8. URL: <http://www.jbks.ru/archive/issue-6/article-8>.
- [53] DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, Mangal D, Yu KH, Yeo CJ, Calhoun ES, et al. Oncogene-induced Nrf2 transcription promotes ROS

- detoxification and tumorigenesis. *Nature*. 2011;475(7354):106–109. doi:10.1038/nature10189.
- [54] Cho HJ, Kang JH, Kwak JY, Lee TS, Lee IS, Park NG, Nakajima H, Magae J, Chang YC. Ascofuranone suppresses PMA-mediated matrix metalloproteinase-9 gene activation through the Ras/Raf/MEK/ERK- and Ap1-dependent mechanisms. *Carcinogenesis*. 2007;28(5):1104–1110.
- [55] Yoshioka K, Deng T, Cavigelli M, Karin M. Antitumor promotion by phenolic antioxidants: inhibition of AP-1 activity through induction of Fra expression. *Proc Natl Acad Sci USA*. 1995;92(11):4972–4976.
- [56] Jin C, Li H, Ugai H, Murata T, Yokoyama KK. Transcriptional regulation of the c-jun gene by AP-1 repressor protein JDP2 during the differentiation of F9 cells. *Nucleic Acids Res Suppl*. 2002;1(2):97–98.
- [57] Borde-Chiché P, Diederich M, Morceau F, Wellman M, Dicato M. Phorbol ester responsiveness of the glutathione S-transferase P1 gene promoter involves an inducible c-jun binding in human K562 leukemia cells. *Leuk Res*. 2001;25(3):241–247.
- [58] Suzuki T, Kelly VP, Motohashi H, Nakajima O, Takahashi S, Nishimura S, Yamamoto M. Deletion of the selenocysteine tRNA gene in macrophages and liver results in compensatory gene induction of cytoprotective enzymes by Nrf2. *J Biol Chem*. 2008;283(4):2021–2030.
- [59] Carvalho AN, Marques C, Guedes RC, Castro-Caldas M, Rodrigues E, van Horsen J, Gama MJ. S-Glutathionylation of Keap1: a new role for glutathione S-transferase pi in neuronal protection. *FEBS Lett*. 2016;590(10):1455–1466. doi:10.1002/1873-3468.12177.
- [60] Sun Z, Wu T, Zhao F, Lau A, Birch CM, Zhang DD. KPNA6 (Importin α 7)-mediated nuclear import of Keap1 represses the Nrf2-dependent antioxidant response. *Mol Cell Biol*. 2011;31(9):1800–1811. doi:10.1128/MCB.05036-11.
- [61] Dickinson DA, Iles KE, Zhang H, Blank V, Forman HJ. Curcumin alters EpRE and AP-1 binding complexes and elevates glutamate-cysteine ligase gene expression. *FASEB J*. 2003;17(3):473–475.
- [62] Pendyala S, Natarajan V. Redox regulation of Nox proteins. *Respir Physiol Neurobiol*. 2010;174(3):265–271. doi:10.1016/j.resp.2010.09.016.
- [63] Pendyala S, Moitra J, Kalari S, Kleeberger SR, Zhao Y, Reddy SP, Garcia JG, Natarajan V. Nrf2 regulates hyperoxia-induced Nox4 expression in human lung endothelium: identification of functional antioxidant response elements on the Nox4 promoter. *Free Radic Biol Med*. 2011;50(12):1749–1759. doi:10.1016/j.freeradbiomed.2011.03.022.

- [64] Shiose A, Kuroda J, Tsuruya K, Hirai M, Hirakata H, Naito S, Hattori M, Sakaki Y, Sumimoto H. A novel superoxide-producing NAD(P)H oxidase in kidney. *J Biol Chem.* 2001;276(2):1417–1423.
- [65] Kuroda J, Nakagawa K, Yamasaki T, Nakamura K, Takeya R, Kuribayashi F, Imajoh-Ohmi S, Igarashi K, Shibata Y, Sueishi K, Sumimoto H. The superoxide-producing NAD(P)H oxidase Nox4 in the nucleus of human vascular endothelial cells. *Genes Cells.* 2005;10(12):1139–1151.
- [66] Van Buul JD, Fernandez-Borja M, Anthony EC, Hordijk PL. Expression and localization of NOX2 and NOX4 in primary human endothelial cells. *Antioxid Redox Signal.* 2005;7(3–4):308–317.
- [67] Bánfi B, Molnár G, Maturana A, Steger K, Hegedûs B, Demaurex N, Krause KH. A Ca(2+)-activated NADPH oxidase in testis, spleen, and lymph nodes. *J Biol Chem.* 2001;276(40):37594–37601.
- [68] Martyn KD, Frederick LM, von Loehneysen K, Dinanier MC, Knaus UG. Functional analysis of Nox4 reveals unique characteristics compared to other NADPH oxidases. *Cell Signal.* 2006;18(1):69–82.
- [69] Manea A, Manea SA, Gafencu AV, Raicu M, Simionescu M. AP-1-dependent transcriptional regulation of NADPH oxidase in human aortic smooth muscle cells: role of p22phox subunit. *Arterioscler Thromb Vasc Biol.* 2008;28(5):878–885. doi:10.1161/ATVBAHA.108.163592.
- [70] Kim HJ, Jung KJ, Yu BP, Cho CG, Choi JS, Chung HY. Modulation of redox-sensitive transcription factors by calorie restriction during aging. *Mech Ageing Dev.* 2002;123(12):1589–1595.
- [71] Jin DY, Chae HZ, Rhee SG, Jeang KT. Regulatory role for a novel human thioredoxin peroxidase in NF-kappaB activation. *J Biol Chem.* 1997;272(49):30952–30961.
- [72] Schenk H, Klein M, Erdbrügger W, Dröge W, Schulze-Osthoff K. Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF-kappaB and AP-1. *Proc Natl Acad Sci U S A.* 1994;91(5):1672–1676.
- [73] Nordberg J, Arnér ES. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med.* 2001;31(11):1287–1312.
- [74] Wu JX, Carpenter PM, Gresens C, Keh R, Niman H, Morris JW, Mercola D. The proto-oncogene c-fos is over-expressed in the majority of human osteosarcomas. *Oncogene.* 1990;5(7):989–1000.
- [75] Bohmann D, Bos TJ, Admon A, Nishimura T, Vogt PK, Tjian R. Human proto-oncogene c-jun encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. *Science.* 1987;238(4832):1386–1392.

- [76] Jeong HS, Ryoo IG, Kwak MK. Regulation of the expression of renal drug transporters in KEAP1-knockdown human tubular cells. *Toxicol In Vitro*. 2015;29(5):884–892. doi:10.1016/j.tiv.2015.03.013.
- [77] Rao S, Tortola L, Perlot T, Wirnsberger G, Novatchkova M, Nitsch R, Sykacek P, Frank L, Schramek D, Komnenovic V et al. A dual role for autophagy in a murine model of lung cancer. *Nat Commun*. 2014;5:3056. doi:10.1038/ncomms4056.
- [78] Sankaranarayanan K, Jaiswal AK. Nrf3 negatively regulates antioxidant-response element-mediated expression and antioxidant induction of NAD(P)H:quinone oxidoreductase1 gene. *J Biol Chem*. 2004;279(49):50810–50817.
- [79] Chevillard G, Blank V. NFE2L3 (NRF3): the Cinderella of the Cap'n'Collar transcription factors. *Cell Mol Life Sci*. 2011;68(20):3337–3348. doi:10.1007/s00018-011-0747-x.
- [80] Derjuga A, Gourley TS, Holm TM, Heng HH, Shivdasani RA, Ahmed R, Andrews NC, Blank V. Complexity of CNC transcription factors as revealed by gene targeting of the Nrf3 locus. *Mol Cell Biol*. 2004;24(8):3286–3294.
- [81] Zhang Y, Kobayashi A, Yamamoto M, Hayes JD. The Nrf3 transcription factor is a membrane-bound glycoprotein targeted to the endoplasmic reticulum through its N-terminal homology box 1 sequence. *J Biol Chem*. 2009;284(5):3195–3210. doi:10.1074/jbc.M805337200.
- [82] Pepe AE, Xiao Q, Zampetaki A, Zhang Z, Kobayashi A, Hu Y, Xu Q. Crucial role of nrf3 in smooth muscle cell differentiation from stem cells. *Circ Res*. 2010;106(5):870–879. doi:10.1161/CIRCRESAHA.109.211417.
- [83] Burton GJ, Jauniaux E. Oxidative stress. *Best Pract Res Clin Obstet Gynaecol*. 2011;25(3):287–299. doi:10.1016/j.bpobgyn.2010.10.016.
- [84] Chepelev NL, Bennitz JD, Huang T, McBride S, Willmore WG. The Nrf1 CNC-bZIP protein is regulated by the proteasome and activated by hypoxia. *PLoS One*. 2011;6(12):e29167. doi:10.1371/journal.pone.0029167.
- [85] Lee OH, Jain AK, Papusha V, Jaiswal AK. An auto-regulatory loop between stress sensors INrf2 and Nrf2 controls their cellular abundance. *J Biol Chem*. 2007;282(50):36412–36420.
- [86] Kaspar JW, Niture SK, Jaiswal AK. Nrf2:INrf2 (Keap1) signaling in oxidative stress. *Free Radic Biol Med*. 2009;47(9):1304–1309. doi:10.1016/j.freeradbiomed.2009.07.035.
- [87] Piantadosi CA, Carraway MS, Babiker A, Suliman HB. Heme oxygenase-1 regulates cardiac mitochondrial biogenesis via Nrf2-mediated transcriptional control of nuclear respiratory factor-1. *Circ Res*. 2008;103(11):1232–1240. doi:10.1161/01.RES.0000338597.71702.ad.

- [88] Jain AK, Jaiswal AK. GSK-3 β acts upstream of Fyn kinase in regulation of nuclear export and degradation of NF-E2 related factor 2. *J Biol Chem*. 2007;282(22):16502–16510.
- [89] Niture SK, Kaspar JW, Shen J, Jaiswal AK. Nrf2 signaling and cell survival. *Toxicol Appl Pharmacol*. 2010;244(1):37–42. doi:10.1016/j.taap.2009.06.009.
- [90] Liu GH, Qu J, Shen X. NF-kappaB/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. *Biochim Biophys Acta*. 2008;1783(5):713–727. doi:10.1016/j.bbamcr.2008.01.002.
- [91] Lee DF, Kuo HP, Liu M, Chou CK, Xia W, Du Y, Shen J, Chen CT, Huo L, Hsu MC et al. KEAP1 E3 ligase-mediated downregulation of NF-kappaB signaling by targeting IKK β . *Mol Cell*. 2009;36(1):131–140. doi:10.1016/j.molcel.2009.07.025.
- [92] Hoshino H, Kobayashi A, Yoshida M, Kudo N, Oyake T, Motohashi H, Hayashi N, Yamamoto M, Igarashi K. Oxidative stress abolishes leptomycin B-sensitive nuclear export of transcription repressor Bach2 that counteracts activation of Maf recognition element. *J Biol Chem*. 2000;275(20):15370–15376.
- [93] Ishikawa M, Numazawa S, Yoshida T. Redox regulation of the transcriptional repressor Bach1. *Free Radic Biol Med*. 2005;38(10):1344–52.
- [94] Klaassen CD, Reisman SA. Nrf2 the rescue: effects of the antioxidative/electrophilic response on the liver. *Toxicol Appl Pharmacol*. 2010;244(1):57–65. doi:10.1016/j.taap.2010.01.013.
- [95] Zhong JL, Raval C, Edwards GP, Tyrrell RM. A role for Bach1 and HO-2 in suppression of basal and UVA-induced HO-1 expression in human keratinocytes. *Free Radic Biol Med*. 2010;48(2):196–206. doi:10.1016/j.freeradbiomed.2009.10.037.
- [96] Dhakshinamoorthy S, Jain AK, Bloom DA, Jaiswal AK. Bach1 competes with Nrf2 leading to negative regulation of the antioxidant response element (ARE)-mediated NAD(P)H:quinone oxidoreductase 1 gene expression and induction in response to antioxidants. *J Biol Chem*. 2005;280(17):16891–16900.
- [97] Kaspar JW, Jaiswal AK. Antioxidant-induced phosphorylation of tyrosine 486 leads to rapid nuclear export of Bach1 that allows Nrf2 to bind to the antioxidant response element and activate defensive gene expression. *J Biol Chem*. 2010;285(1):153–162. doi:10.1074/jbc.M109.040022.
- [98] Suzuki H, Tashiro S, Hira S, Sun J, Yamazaki C, Zenke Y, Ikeda-Saito M, Yoshida M, Igarashi K. Heme regulates gene expression by triggering Crm1-dependent nuclear export of Bach1. *EMBO J*. 2004;23(13):2544–2553.
- [99] Vriend J, Reiter RJ. The Keap1-Nrf2-antioxidant response element pathway: a review of its regulation by melatonin and the proteasome. *Mol Cell Endocrinol*. 2015;401:213–220. doi:10.1016/j.mce.2014.12.013.

- [100] Thomsen MK, Bakiri L, Hasenfuss SC, Hamacher R, Martinez L, Wagner EF. JUNB/AP-1 controls IFN- γ during inflammatory liver disease. *J Clin Invest.* 2013;123(12):5258–5268. doi:10.1172/JCI70405.
- [101] Realini C, Dubiel W, Pratt G, Ferrell K, Rechsteiner M. Molecular cloning and expression of a gamma-interferon-inducible activator of the multicatalytic protease. *J Biol Chem.* 1994;269(32):20727–20732.
- [102] Ahn JY, Tanahashi N, Akiyama K, Hisamatsu H, Noda C, Tanaka K, Chung CH, Shibmura N, Willy PJ, Mott JD, et al. Primary structures of two homologous subunits of PA28, a gamma-interferon-inducible protein activator of the 20S proteasome. *FEBS Lett.* 1995;366(1):37–42.
- [103] Cheng C, Li W, Zhang Z, Yoshimura S, Hao Q, Zhang C, Wang Z. MicroRNA-144 is regulated by activator protein-1 (AP-1) and decreases expression of Alzheimer disease-related a disintegrin and metalloprotease 10 (ADAM10). *J Biol Chem.* 2013;288(19):13748–13761. doi:10.1074/jbc.M112.381392.
- [104] Sangokoya C, Telen MJ, Chi JT. microRNA miR-144 modulates oxidative stress tolerance and associates with anemia severity in sickle cell disease. *Blood.* 2010;116(20):4338–4348. doi:10.1182/blood-2009-04-214817.
- [105] Papp D, Lenti K, Módos D, Fazekas D, Dúl Z, Türei D, Földvári-Nagy L, Nussinov R, Csermely P, Korcsmáros T. The NRF2-related interactome and regulome contain multifunctional proteins and fine-tuned autoregulatory loops. *FEBS Lett.* 2012;586(13):1795–1802. doi:10.1016/j.febslet.2012.05.016.
- [106] Zolotukhin PV, Belanova AA, Prazdnova EV, Mazanko MS, Batiushin MM, Chmyhalo VK, Chistyakov VA. Mitochondria as a signaling Hub and target for phenoptosis shutdown. *Biochemistry (Mosc).* 2016;81(4):329–337. doi:10.1134/S0006297916040039.
- [107] Miyamoto N, Izumi H, Miyamoto R, Kondo H, Tawara A, Sasaguri Y, Kohno K. Quercetin induces the expression of peroxiredoxins 3 and 5 via the Nrf2/NRF1 transcription pathway. *Invest Ophthalmol Vis Sci.* 2011;52(2):1055–1063. doi:10.1167/iov.10-5777.
- [108] Klinge CM. Estrogenic control of mitochondrial function and biogenesis. *J Cell Biochem.* 2008;105(6):1342–1351. doi:10.1002/jcb.21936.
- [109] Webb P, Nguyen P, Valentine C, Lopez GN, Kwok GR, McInerney E, Katzenellenbogen BS, Enmark E, Gustafsson JA, Nilsson S et al. The estrogen receptor enhances AP-1 activity by two distinct mechanisms with different requirements for receptor transactivation functions. *Mol Endocrinol.* 1999;13(10):1672–1685.
- [110] Ross D, Kepa JK, Winski SL, Beall HD, Anwar A, Siegel D. NAD(P)H:quinone oxidoreductase 1 (NQO1): chemoprotection, bioactivation, gene regulation and genetic polymorphisms. *Chem Biol Interact.* 2000;129(1–2):77–97.
- [111] Dahlman-Wright K, Qiao Y, Jonsson P, Gustafsson JÅ, Williams C, Zhao C. Interplay between AP-1 and estrogen receptor α in regulating gene expression and proliferation

- networks in breast cancer cells. *Carcinogenesis*. 2012;33(9):1684–1691. doi:10.1093/carcin/bgs223
- [112] Lo SC, Hannink M. PGAM5 tethers a ternary complex containing Keap1 and Nrf2 to mitochondria. *Exp Cell Res*. 2008;314(8):1789–1803. doi:10.1016/j.yexcr.2008.02.014.
- [113] Lu W, Sun J, Yoon JS, Zhang Y, Zheng L, Murphy E, Mattson MP, Lenardo MJ. Mitochondrial Protein PGAM5 Regulates Mitophagic Protection against Cell Necroptosis. *PLoS One*. 2016;11(1):e0147792. doi:10.1371/journal.pone.0147792. eCollection 2016.
- [114] Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, Springer W. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol*. 12(2):119–131. doi:10.1038/ncb2012.
- [115] Fratta Pasini A, Albiero A, Stranieri C, Cominacini M, Pasini A, Mozzini C, Vallerio P, Cominacini L, Garbin U. Serum oxidative stress-induced repression of Nrf2 and GSH depletion: a mechanism potentially involved in endothelial dysfunction of young smokers. *PLoS One*. 2012;7(1):e30291. doi:10.1371/journal.pone.0030291.
- [116] Sirianni R, Nogueira E, Bassett MH, Carr BR, Suzuki T, Pezzi V, Andò S, Rainey WE. The AP-1 family member FOS blocks transcriptional activity of the nuclear receptor steroidogenic factor 1. *J Cell Sci*. 2010;123(Pt 22):3956–3965. doi:10.1242/jcs.055806.
- [117] Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. *Proc Natl Acad Sci U S A*. 1996;93(25):14960–14965.
- [118] Ohtsubo T, Kamada S, Mikami T, Murakami H, Tsujimoto Y. Identification of NRF2, a member of the NF-E2 family of transcription factors, as a substrate for caspase-3(-like) proteases. *Cell Death Differ*. 1999;6(9):865–872.
- [119] Shukla SJ, Huang R, Simmons SO, Tice RR, Witt KL, Vanleer D, Ramabhadran R, Austin CP, Xia M. Profiling environmental chemicals for activity in the antioxidant response element signaling pathway using a high throughput screening approach. *Environ Health Perspect*. 2012;120(8):1150–1156. doi:10.1289/ehp.1104709.
- [120] Belanova AA, Smirnov DS, Makarenko MS, Belousova MM, Aleksandrova AA, Soldatov AV, Zolotukhin PV. Individual expression features of GPX2, NQO1 and SQSTM1 transcript variants induced by hydrogen peroxide treatment in HeLa cells. *Genetics and molecular biology*. 2015. Publication in progress, major revision submitted.
- [121] Crunkhorn S. Deal watch: Abbott boosts investment in NRF2 activators for reducing oxidative stress. *Nat Rev Drug Discov*. 2012;11(2):96. doi:10.1038/nrd36