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REVIEW

NFE2L3 (NRF3): the Cinderella of the Cap'n'Collar transcription factors

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Abstract NFE2L3 [Nuclear factor (erythroid-derived 2)-like 3] or NRF3, a member of the Cap'n'Collar (CNC) family, is a basic-region leucine zipper (bZIP) transcription factor that was first identified over 10 years ago. Contrary to its extensively studied homolog NFE2L2 (NRF2), the regulation and function of the NFE2L3 protein have not yet attracted as much attention. Nevertheless, several recent reports have now shed light on the possible roles of NFE2L3. Structural and biochemical studies revealed a series of domains and modifications that are critical for its cellular regulation. The control of the subcellular localization of NFE2L3 appears to be essential for understanding its role in various cellular processes. Importantly, newer studies provide fascinating insights linking NFE2L3 to differentiation, inflammation, and carcinogenesis. Here, we present an overview of the current level of knowledge of NFE2L3 transcription factor biology in humans and mice. From being the Cinderella of the CNC transcription factors for many years, NFE2L3 may now rapidly come into its own.

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Introduction

The Cap'n'Collar (CNC) proteins are a subgroup of basic region-leucine zipper (bZIP) transcription factors conserved among birds, insects, worms, fish, and mammals but absent in plants and fungi [1]. Since the discovery in 1989 of the first CNC transcription factor NFE2 (Nuclear Factor-Erythroid derived 2), also known as p45 NFE2 [2], several other members have been identified. The CNC family includes the *Drosophila* CNC1 protein [3], *Caenorhabditis elegans* Skn-1 [4], and vertebrate NFE2L1 (Nuclear factor erythroid 2 related factor-1) also known as NRF1/LCRF/TCF11 [5–7], NFE2L2 (or NRF2) [8], NFE2L3 (or NRF3) [9–11], as well as the more distantly related BACH1 [12] and BACH2 [12] proteins.

Members of the CNC family are characterized by a highly conserved 43-amino-acid region (Figs. 1, 2, 3), referred to as the CNC domain, which contributes to the unique DNA-binding specificity of these transcription factors [13, 14]. CNC proteins also contain a basic region-leucine zipper motif (bZIP) consisting of a region rich in basic residues conferring DNA-binding activity, followed by six heptad repeats of hydrophobic residues forming a leucine zipper motif, which acts as a dimerization domain (Figs. 1, 2, 3) [15]. The DNA-binding domain includes a nuclear localization signal, which has been shown to be functional in human p45 NFE2 [16], NFE2L2 [17], and BACH2 [18]. BACH1 and BACH2 proteins are characterized by the presence of a BTB (Broad complex, Tramtrack, Bric-a-brac) domain required for protein–protein

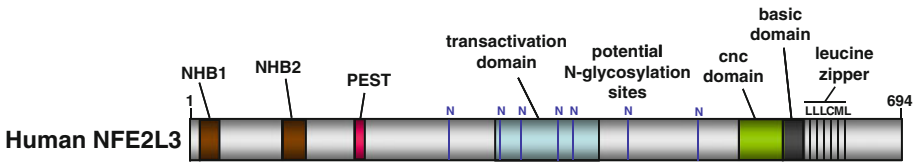

Fig. 1 Structure of the human NFE2L3 transcription factor. *NHB1* N-terminal homology box 1, *NHB2* N-terminal homology box 2, *PEST* PEST domain, *N* potential *N*-glycosylation sites, transactivation domain, transactivation domain as defined by Gal4-luciferase reporter studies, *CNC domain* Cap'n'Collar homology domain, *basic domain* basic DNA-binding domain, *leucine zipper* leucine zipper dimerization domain

interaction and facilitating homo- and/or hetero-dimer formation [12, 19–21]. CNC factors function as obligate heterodimers by complexing with small Maf (musculoaponeurotic fibrosarcoma) MAFG, MAFK, and MAFF [9, 22–28] and jun proteins [29, 30] for DNA binding. The resulting complexes bind to NFE2 (Nuclear Factor-Erythroid 2)-, MARE (Maf recognition element)-, ARE (antioxidant response element)- and StreB (stress-response element)/EpRE (electrophile response element)-type DNA binding sites [9, 10, 28, 31–33].

The role and regulation of the CNC member NFE2L2 in oxidative stress and in cancer prevention have been extensively studied and discussed in many reviews [34–59]. Furthermore, several reviews focus on the respective roles of p45 NFE2 [60–64], NFE2L1 [65], BACH1 [66–68], BACH2 [69, 70], and small MAF proteins [26, 28, 71–73]. Here, we provide the first review that focuses specifically on NFE2L3, including recent insights into its biology and its potential relevance to human diseases.

Structure of NFE2L3 gene and protein

The cloning of the human and mouse *NFE2L3* genes was first reported in 1999 [9]. Fluorescent in situ hybridization (FISH) experiments mapped the human *NFE2L3* gene on the chromosome 7p15-p14 [9], whereas the mouse *Nfe2l3* is located on chromosome 6B3 [11]. In both species, the *NFE2L3* gene maps near the *HOXA* gene cluster. This is similar to the genetic loci of *p45 NFE2*, *NFE2L1*, and *NFE2L2*, which map near the *HOXC*, *HOXB*, and *HOXD* genes, respectively [9, 74]. These observations support the idea that *p45 NFE2*, *NFE2L1*, *NFE2L2*, and *NFE2L3* are derived from a single ancestral gene localized in proximity to the ancestral *HOX* cluster and they have diverged to give rise to four closely related transcription factors through chromosome duplication [9]. In contrast, the *BACH1* and *BACH2* genes seem to derive from another duplication since both genes are not associated with any *HOX* cluster. This observation could explain why *Bach1* and *Bach2* have functions distinct from the other CNC transcription factors.

The human *NFE2L3* transcript encodes a 694-amino-acid protein, whereas mouse *Nfe2l3* mRNA gives rise to a 660-amino-acid protein (Fig. 3) [9, 10]. Bioinformatics analysis of the NFE2L3 proteins among different species from zebrafish to human indicates a high degree of conservation through evolution of its key domains including the NHB1 (N-terminal homology box 1) domain, the NHB2 (N-terminal homology box 2) domain [75], the CNC domain, the basic region and the leucine zipper domains, supporting the importance of these domains for NFE2L3 functions (Figs. 1, 3). Intriguingly, human and mouse NFE2L3 proteins share only approximately 68% homology (Fig. 3), which is significantly less than among the orthologues of other CNC family members with 89, 97, and 80% overall identities for p45 NFE2, NFE2L1, and NFE2L2, respectively [9]. This suggests that the human and mouse NFE2L3 proteins may have acquired relatively more distinct functions when compared to the other CNC family members.

NFE2L3 expression in tissues and cells

Expression levels of NFE2L3 have been studied in both tissues and cells of human and mouse origins [9–11, 54, 76–81]. For instance, the highest levels of human NFE2L3 are found in placenta, specifically in chorionic villi from at least week 12 of gestation through term [9, 10]. Its expression has been found in primary placental cytotrophoblasts, but not in placental fibroblasts. In line with these data, *NFE2L3* transcript and protein levels are high in both BeWo and JAR cell lines (Table 1), which are human choriocarcinoma cell lines derived from trophoblastic tumors of the placenta [10, 82]. Besides its expression in placenta, human *NFE2L3* is expressed from intermediate to low levels in a wide variety of other tissues including heart, brain, lung, kidney, pancreas, leukocytes, colon, thymus, and spleen [9]. Barely detectable levels of human *NFE2L3* were observed in human megakaryocytes and erythrocytes [80], whereas no expression was found in testis, prostate, skeletal muscle, and ovary [9]. *NFE2L3* transcripts and/or proteins are expressed in a series of cell lines (Table 1) [9, 10, 75, 81–85]. Expression of *Nfe2l3* in mouse tissues

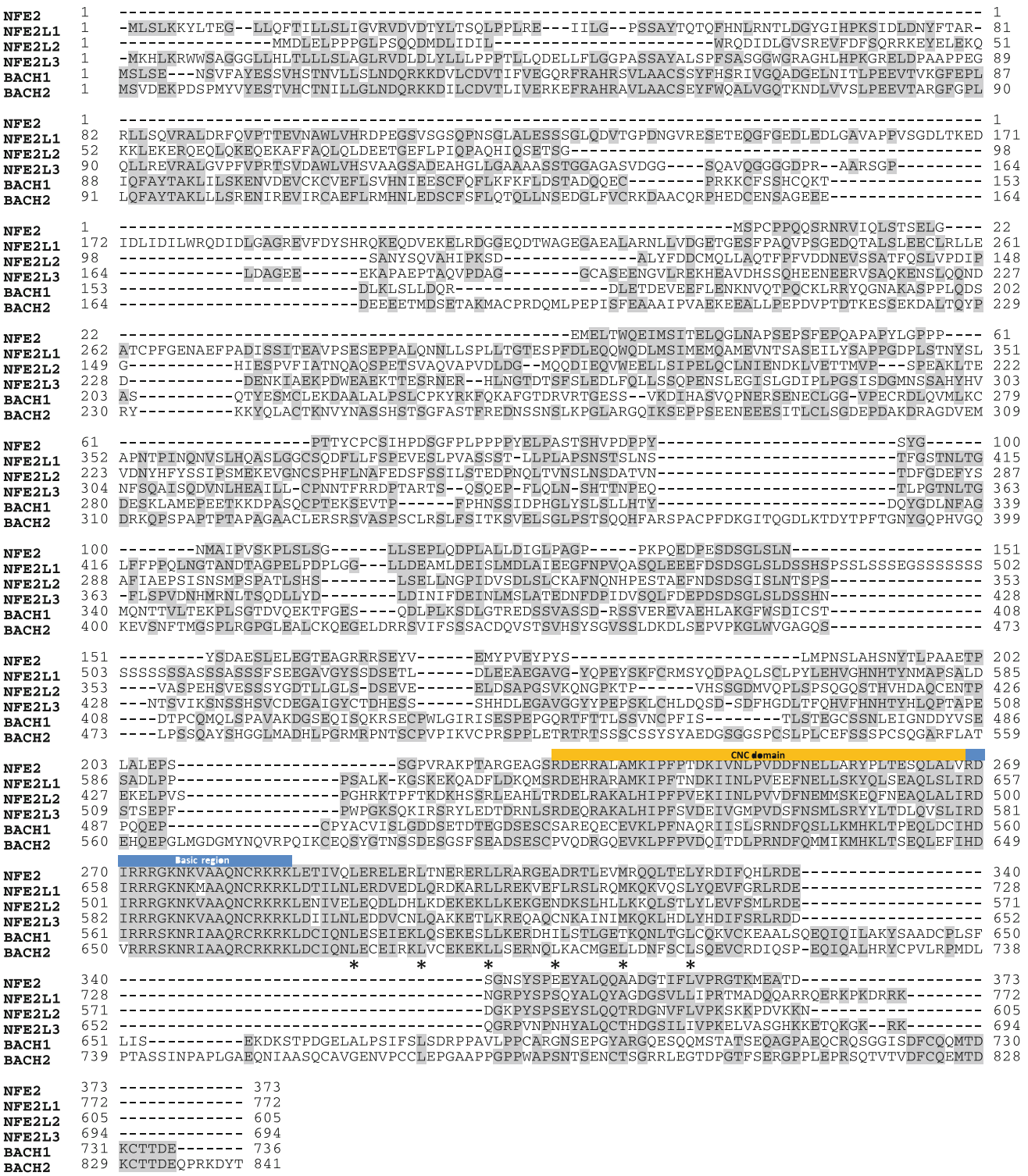

Fig. 2 Comparison of human CNC transcription factor sequences. Alignment of human p45 NFE2, NFE2L1, NFE2L2, NFE2L3, BACH1, and BACH2 protein sequences was performed using ClustalW program [117]. Identical residues are shaded in *grey*. The basic region (in *light blue*) and the CNC domain (in *orange*) are indicated. *Asterisks* indicate leucine zipper residues

is broad with high levels found in thymus, brain, lung, stomach, uterus, placenta, adipose tissue, and testis [11, 76, 78].

The expression pattern of *NFE2L3* was evaluated by in situ hybridization during development of avian embryos and revealed expression in mesodermal derivatives

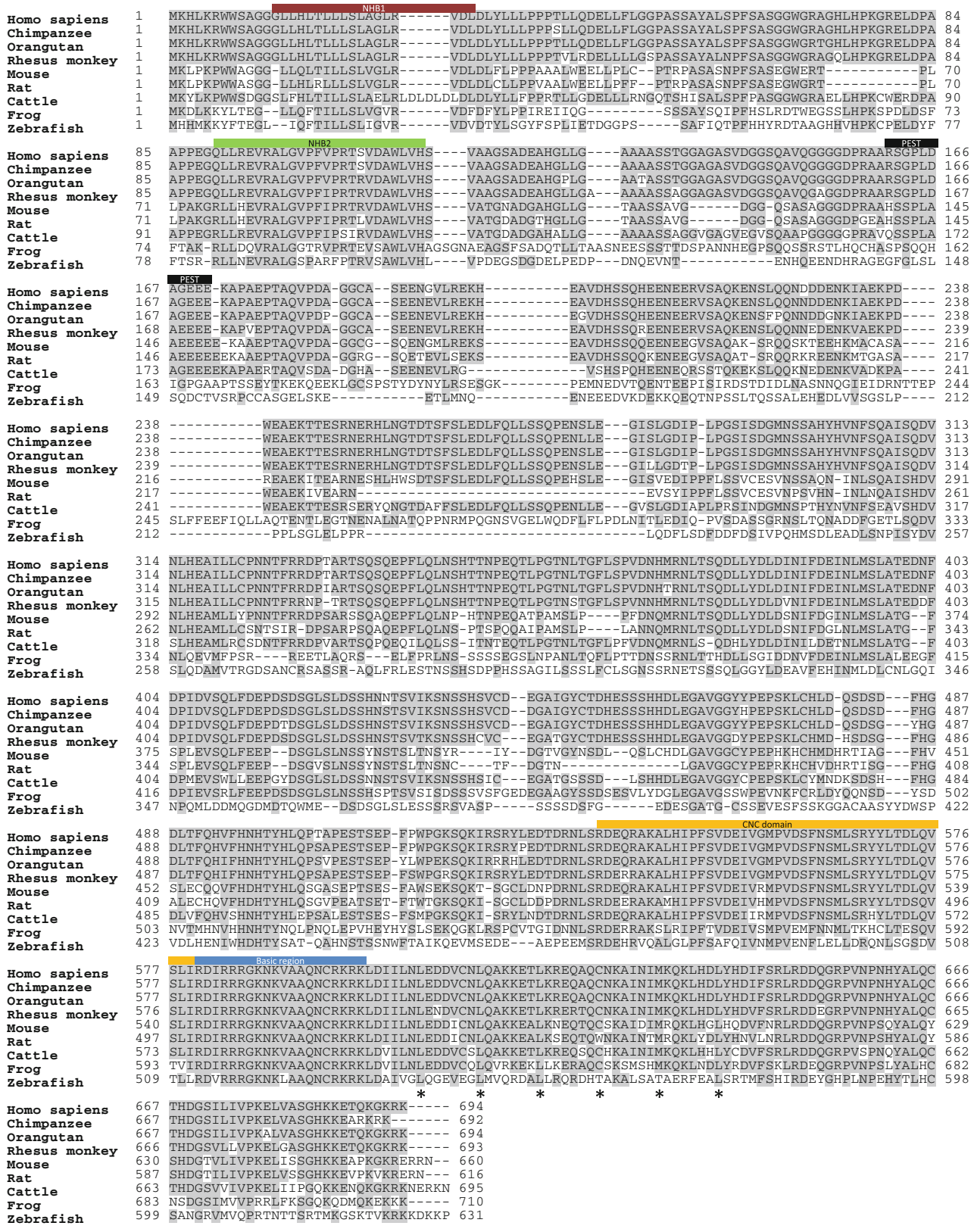

Fig. 3 Cross-species comparison of NFE2L3 sequences. Protein sequences of NFE2L3 from nine different species were aligned using ClustalW program [117]. Identical residues are shaded in *grey*. The basic region (in *light blue)*, the CNC domain (in *orange*), the NHB1 domain (in *purple*), the NHB2 domain (in *light green*) and the PEST motif (in *black*) are indicated. *Asterisks* indicate leucine zipper residues

Table 1 List of human cell lines expressing NFE2L3 at the RNA and/or protein level

Cell lines	NFE2L3 expression	
	Cell/tumor type	References
HL-60	Acute promyelocytic leukemia	[9]
THP-1	Acute monocytic leukemia	[9]
RPMI8226	Myeloma	[9]
U937	Histiocytic lymphoma	[9]
Raji	Burkitt lymphoma	[9]
BALM-2	Burkitt lymphoma	[9]
NAMALWA	Burkitt lymphoma	[9]
HRS	Hodgkin lymphoma	[84]
HL	Hodgkin lymphoma	[83]
BeWo	Choriocarcinoma	[10]
JAR	Choriocarcinoma	[10, 75]
MDA-MB-231	Breast cancer	[82]
MCF-10	Breast cancer	[85]
HaCaT	Keratinocytes (non-tumorigenic)	[81]

including heart, somites, yolk sac, and kidney. Since this was different to the rather ubiquitous expression described for other CNC factors, it was suggested that NFE2L3 may play an earlier, more specific role in target gene regulation in the developing avian embryo [86].

Molecular mechanisms of NFE2L3 transactivation

During the past few years, important progress has been made in the understanding of NFE2L3 transactivation. NFE2L3 was identified as a partner of MafG in a yeast-based in vivo protein–protein interaction screen [10]. Functional studies using a Gal4-luciferase reporter revealed the presence of a potent transactivation domain in the center of human NFE2L3 protein (amino acids 298–399). It was shown that NFE2L3 can transcriptionally activate MARE-driven, β-globin gene expression in QT6 cells [9] as well as ARE-driven, NADPH dehydrogenase quinone 1 (*Nqo1*) gene expression in COS-1 cells [75]. Using the same reporter gene, Zhang and colleagues found that the transactivation capacity of NFE2L3 appeared to be less potent than NFE2L1 or NFE2L2 [75]. In contrast, overexpression of NFE2L3 leads to a repression of both luciferase reporter under the control of the NQO1 ARE as well as endogenous *Nqo1* gene expression in human Hep-G2 cells [87] and mouse embryonic stem cells [79], indicating that NFE2L3 can either activate or repress transcription of its target genes depending on the cellular context. One may hypothesize that these differences are due to the expression levels of NFE2L3 and/or its cofactors.

Complex regulation of NFE2L3 expression

Biochemical studies revealed the existence of at least three differently migrating forms of endogenous NFE2L3, a slow migrating 'A' form, an intermediate 'B' form, and a faster migrating 'C' form [82]. Fractionation studies and immunofluorescence experiments showed that the various NFE2L3 versions are localized in specific subcellular compartments: 'A' is associated with the endoplasmic reticulum, whereas 'B' is mostly cytoplasmic and 'C' is found mainly in the nucleus (Fig. 4) [75, 82]. The 'A', 'B,' and 'C' forms of human NFE2L3 have short half-lives, generally less than 1 h. In accordance with the rapid turnover, a PEST motif has been identified in the NFE2L3 sequence and is conserved across different species (Fig. 3) [75, 82]. This motif has been shown to be functional and to negatively regulate its activity [75]. Studies using different proteasome inhibitors strongly suggest that the three NFE2L3 versions are degraded through the ubiquitin–proteasome pathway (Fig. 4) [82].

What distinguishes the 'A', 'B', and 'C' forms of NFE2L3? Using the N-linked specific deglycosylating enzymes PNGase F and EndoH, it was demonstrated that the 'A' form corresponds to a *N*-glycosylated NFE2L3 protein, whereas the 'B' and 'C' forms are unglycosylated (Fig. 4) [75, 82]. Treatment with both deglycosylation enzymes increases the 'B' form of NFE2L3 indicating that this version of NFE2L3 is indeed the unglycosylated form of 'A'. Accordingly, seven potential sites for N-linked glycosylation were identified in the center portion of the NFE2L3 protein (Fig. 1). The exact nature of the 'C' form of NFE2L3 remains unclear. It has been speculated that it may be a truncated form of NFE2L3 lacking the N-terminal portion of the protein (Fig. 4) [82]. However, the site of cleavage generating the C form is still unidentified and one may hypothesize that this cleavage site is different from the one that releases the signal peptide targeting the protein to the ER. It has been reported that residues 40–75 of mouse NFE2L3, comprising several Site-1 protease recognition sites, control proteolytic processing that produces the 'C' form. Indeed, transactivation activity of NFE2L3 is inhibited by *N*-acetyl-L-leucyl-L-leucyl-L-norleucinal (ALLN), an inhibitor of proteasome activity, which has been shown to block the cleavage activity of the Site-1 protease [75]. However, human NFE2L3 sequence lacks a signal peptidase cleavage site [75], adding another layer of complexity to NFE2L3 regulation. With respect to mouse NFE2L3, a hypothetical model has been proposed that describes possible mechanisms that control its translocation from the ER to the nucleus, and it was also suggested that a deglycosylation event is required [75].

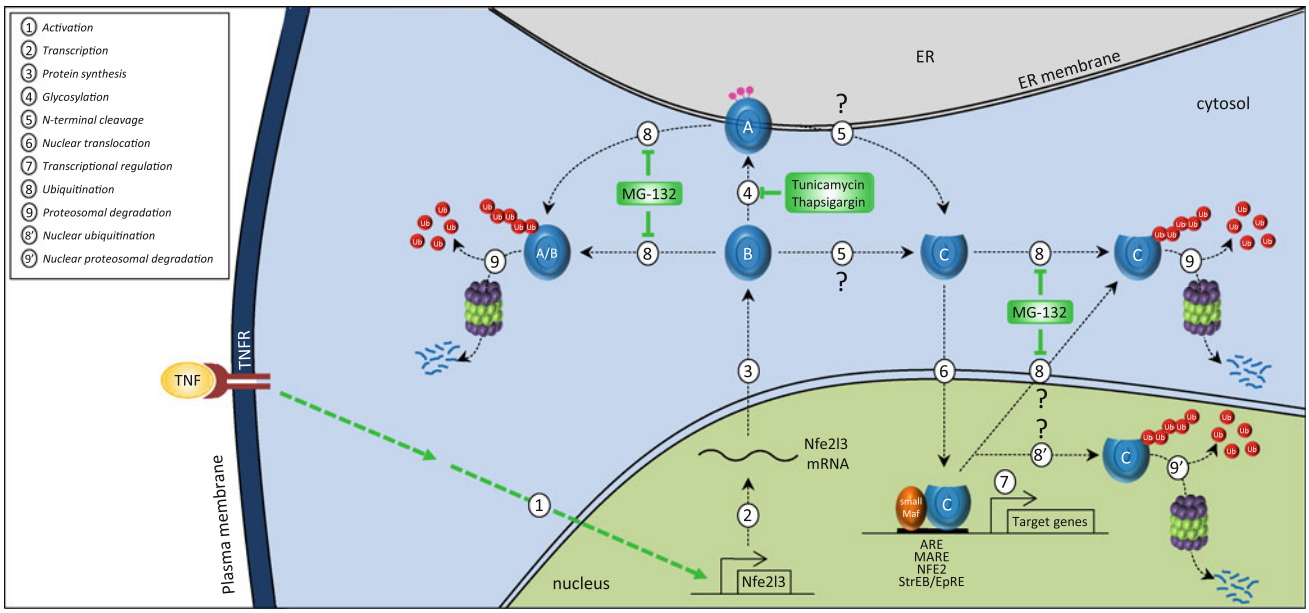

Fig. 4 Proposed model for NFE2L3 regulation. Hypothetical model to describe the cellular regulation of NFE2L3 based on published literature. Once activated by a stimulus (e.g., TNF), transcription of *NFE2L3* gene occurs in the nucleus leading to the production of *NFE2L3* RNA in the nucleus and then to the translation in the cytosol into a 'B' form of NFE2L3 protein. The 'B' form can be targeted to the ER where it is *N*-glycosylated to become the A form of NFE2L3. The 'A' and/or 'B' forms of NFE2L3 is (are) hypothesized to be cleaved at the N-terminal end into a 'C' form, which is mainly found in the nucleus. The 'C' form is considered to be the major active form of NFE2L3 heterodimerizing with small Maf proteins. The resulting heterodimers activate transcription of their target genes through binding to specific DNA-binding sites including ARE, MARE, NFE2, StreB/EpRE sequences. The NFE2L3 'A', 'B', and 'C' forms have short half-lives and are most likely degraded through the ubiquitin–proteasome pathway in the cytosol and/or in the nucleus

The NHB domains: important features of NFE2L3

Comparison between mouse NFE2L1 and NFE2L3 protein sequences revealed two conserved regions called NHB1 and NHB2 domains. The NHB1 domain, part of an ER signal peptide, has been previously shown to be required for ER targeting of the CNC factor NFE2L1 [88–90]. Of interest, the NHB1 domain of NFE2L3 is also present and highly conserved among species (Fig. 2). Recently, it has been reported that the NHB1 domain of mouse NFE2L3 is indeed necessary to target the protein to the ER and to enable the increase of NFE2L3 activity by the ER stressors tunicamycin and brefeldin A [75]. This is in contrast to the human protein, whose levels are decreased in the presence of tunicamycin [82]. The function of the NHB2 sequence of NFE2L3 still remains uncertain, but it may be involved in the control of NFE2L3 activity as well as its post-translational processing within the ER [75].

Mouse knockout models of CNC transcription factors

CNC factor deficient mice

Significant progress in the understanding of the function of the CNC transcription factor family has been made through the generation of knockout mouse models [11, 54, 91–95]. Gene targeting experiments showed that the p45 NFE2 protein is required for megakaryocyte biogenesis [96]. Homozygous *Nfe2l1* null mice die in utero during mid- to late embryonic development due to a non-cell autonomous defect in definitive erythropoiesis [92, 97], whereas *Nfe2l2* is dispensable for mouse development [95]. *Bach1* null mice develop normally and are fertile [94]. Analysis of *Bach2* null animals revealed that this factor is a key regulator of antibody class switching in B cells [93].

Analysis of Nfe2l3-deficient mice

Until recently, the physiological role of NFE2L3 remained elusive. To gain insights into the in vivo roles of NFE2L3 protein, mice deficient for this transcription factor gene were independently generated in two laboratories [11, 54]. *Nfe2l3* null mice develop and grow normally under non-challenging conditions. No differences were observed between wild-type and *Nfe2l3*$^{-/-}$ mice with respect to several blood parameters including red blood cell count, white blood cell count, erythrocyte cellular index as well as blood chemistries including glucose, blood urea, nitrogen, cholesterol, triglycerides, iron, and bilirubin [11]. Mice were infected with acute lymphocytic choriomeningitis virus and no difference was found between wild-type and

Nfe2l3 null mice in the number of virus-specific CD8 and CD4 T cells as well as B-lymphocyte response [11].

To further investigate functional redundancy among CNC proteins, *Nfe2l3* null mice were crossed with *Nfe2l2* and/or *p45 Nfe2* deficient mice. *Nfe2l3*$^{-/-}$:*Nfe2l2*$^{-/-}$ and *Nfe2l3*$^{-/-}$:*p45 Nfe2* mice develop normally and exhibit survival rates corresponding to the expected Mendelian ratio [54] and do not exhibit defects beyond those seen with the loss of *p45 Nfe2* alone [11]. Triple-compound knockout *p45 Nfe2*$^{-/-}$:*Nfe2l2*$^{-/-}$:*Nfe2l3*$^{-/-}$ mice were also generated and unexpectedly some survive to adulthood, suggesting that the CNC protein NF2L1 and/or other regulatory factors may compensate for the combined absence of p45 NFE2, NFE2L2, and NFE2L3 [11].

Linking NFE2L3 and carcinogenesis: in vivo evidence from mouse model

Recent in vivo data linked the NFE2L3 transcription factor to protection against lymphomagenesis [98]. *Nfe2l3*$^{-/-}$ mice exposed for four consecutive weeks to benzo[a]pyrene (B[a]P), a carcinogen present in tobacco smoke [99], exhibited significantly increased mortality compared to wild-type animals. Thirty-two percent of B[a]P-treated *Nfe2l3*$^{-/-}$ mice developed lymphoma, whereas only 6% of wild-type mice did. Pathological analyses of affected tissue sections revealed a high incidence (21%) of T-cell lymphoblastic lymphoma in B[a]P-treated *Nfe2l3*$^{-/-}$ mice. In line with this data, high expression of *Nfe2l3* transcripts was observed in the normal thymus of both human and mouse origin [9, 11]. These findings strongly suggest a protective role for NFE2L3 transcription factor in carcinogen-induced lymphomagenesis.

A series of genechip array data further hints at a possible role of NFE2L3 in various malignancies. Human *NFE2L3* transcript levels are increased in different types of lymphoma including Hodgkin lymphoma [83, 84], non-Hodgkin cell lineages [83], as well as Mantle cell lymphoma specimens [100–103]. In addition to its overexpression in human lymphoma samples, *NFE2L3* mRNA levels were found to be upregulated in human breast cancer cells [85] and testicular carcinoma tissue samples [104, 105]. Nevertheless, these gene profiling results strictly reflect mRNA levels and it is not known whether the *NFE2L3* transcripts induced in these human samples code for functional or mutated versions of the transcription factor.

NFE2L3 and inflammation

In addition to its function in carcinogenesis, there is evidence that NFE2L3 may have a role in inflammation. *Nfe2l3* null mice have been shown to be highly sensitive to exposure to the antioxidant butylated hydroxytoluene (BHT), which provokes acute lung injury after a single administration in mice [106]. BHT-treated *Nfe2l3*$^{-/-}$ mice exhibit respiratory distress as well as increased body weight loss when compared to their wild-type counterparts [78]. At the molecular level, BHT treatment decreases both *Nfe2l1* and *Nfe2l3* transcripts in the lung of wild-type mice, whereas *Nfe2l2* mRNA levels are upregulated under similar conditions. In addition, basal gene expression of the gene coding for Pparγ2 (peroxisome proliferator-activated receptor gamma 2), a protein possessing anti-inflammatory properties, was found to be increased in white adipose tissue and lung of *Nfe2l3*-deficient mice, suggesting a role for this factor in its transcriptional regulation [107]. Contrary to the highly toxic BHT, butylated hydroxyanisole (BHA), another phenolic antioxidant, is well tolerated in mice [108]. Exposure of wild-type animals to BHA induces hepatic *Nqo1* gene expression; similar results were observed in BHA-treated *Nfe2l3*-deficient mice [54], indicating that expression of *Nfe2l3* gene is dispensable, at least in liver, for the regulation of *Nqo1* gene expression. This may be due to the fact that *Nfe2l3* is not (or only minimally) expressed in the liver [11]. In wound healing experiments, it has been shown that *Nfe2l2*$^{-/-}$ mice exhibit increased infiltration of macrophages, resulting in prolonged inflammation [81]. Furthermore, an upregulation of *Nfe2l3* mRNA levels was reported in both unwounded and early wounded skin of *Nfe2l2* null animals, suggesting that NFE2L3 may compensate in this tissue for the absence of NFE2L2. In addition, the authors proposed that, as demonstrated for NFE2L2, NFE2L3 may be under the control of KGF (Keratinocyte growth factor) in the human keratinocyte cell line HaCaT [81].

Additional support for the importance of NFE2L3 regulation following specific inflammatory cytokines comes from gene regulation studies. It was found that transcripts as well as protein levels of NFE2L3 are upregulated by the cytokine TNF in JAR choriocarcinoma cells (Fig. 4) [10], while genechip array studies showed that interferon-γ increases *NFE2L3* mRNA levels in human uterine endothelial cells [109]. However, not all inflammatory cytokines induce expression of *NFE2L3* transcripts since interleukin 1beta does not modulate *NFE2L3* mRNA levels in human myometrial PHM1-31 cells.

Target genes of NFE2L3

Identification of NFE2L3 target genes is essential for a full understanding of its roles, its regulation, and to identify the pathways regulated by NFE2L3. Although several potential target genes of NFE2L3 have been reported in the literature, no clear evidence has been provided for an in vivo

relevance. For instance, reporter assay studies identified NFE2L3 as a negative regulator of the *Prdx6* promoter in human pulmonary A549 cells [79, 110]. Hence, it is likely that *Prdx6* is a target gene of NFE2L3, at least in an in vitro context. Another candidate gene regulated by NFE2L3 may be *Nqo1*, since, overexpression of NFE2L3 leads to decreased *Nqo1*expression in Hep-G2 [87] and smooth muscle cells [79]. The repression requires DNA binding of NFE2L3 to the ARE of the *Nqo1*promoter, but not the transcriptional activation domain of NFE2L3 [87]. Besides the *Nqo1* gene, it was also proposed that the smooth muscle cell marker genes *SMαA*, *SM22α*, *calponin* and *Nox4* could be potential targets of NFE2L3 [79]. Finally, the *Pparg2* gene and more unpredictably the *Nfe2l2* gene may be targets of NFE2L3 as recently suggested [78]. However, further experiments are required to confirm that all genes mentioned above are genuine targets of NFE2L3 in vivo.

Novel possible avenues for NFE2L3 function

A few recent reports shed light on possible novel functions of the NFE2L3 protein. For instance, NFE2L3 was proposed to be used as a stemness marker gene since its mRNA levels had the highest fold change (>300-fold change) when comparing rhesus macaque fibroblasts to pluripotent stem cells [111, 112]. This interesting result was confirmed by other laboratories using rhesus monkey embryonic stem cells transduced with specific transcription factors [113, 114].

Additionally, Pepe and colleagues [79] described a role for NFE2L3 in mouse smooth muscle cell (SMC) differentiation from stem cells. Unfortunately, it is not clear whether the effects observed in smooth muscle cells are due to the 'A', 'B', or 'C' forms of NFE2L3 as the authors observed only one form, for which the molecular weight was not specified, as opposed to the multiple forms of NFE2L3 seen in earlier reports [75, 82].

Genome-wide association studies recently identified NFE2L3 locus as potentially associated with human endometriosis, a common gynecological disease [115] and with waist-to-hip ratio, a measurement of obesity and an indicator for developing serious health conditions including diabetes [116]. Although somewhat unanticipated, these findings will require further attention in order to understand other potential hidden facets of NFE2L3.

Conclusions and perspectives

Recent studies provide exciting novel data that advance our understanding of the NFE2L3 transcription factor at the cellular level as well as in vivo. Indeed, several reports demonstrate that NFE2L3 is a stringently regulated and a post-translationally modified transcription factor [9–11, 75, 78, 79, 82, 86, 87]. Specifically, glycosylation of NFE2L3 appears to be an important modification targeting one form of the transcription factor to the endoplasmic reticulum. One cannot exclude that NFE2L3 protein undergoes additional post-translational modifications such as phosphorylation, sumoylation, and/or ubiquitination. Although it is hypothesized that the nuclear NFE2L3 is cleaved at the N-terminal end, the specific cleavage site still remains unknown [75, 82].

It would certainly be of interest to identify the regulators of NFE2L3 expression. Besides the proinflammatory cytokine TNF and interferon-γ, no other cellular stimuli have yet been described as controlling NFE2L3 levels. In addition, it will be a major challenge to identify the pathways that regulate the activity of this transcription factor and to determine the role played by NFE2L3 in the ER.

Our understanding of NFE2L3 biology has been improved through the analysis of cellular models and the generation of mice deficient in this transcription factor. Absence of NFE2L3 renders the mice susceptible to carcinogen-induced lymphomagenesis [98], suggesting a possible role for NFE2L3 in the regulation of T cells. The fact that NFE2L3 transcripts have been found to be elevated in many different cancer types in gene expression profiling studies [83–85, 100–105] suggests a link of NFE2L3 to human cancer. Hence, NFE2L3 and associated pathways may represent novel potential targets for therapeutic treatment of cancer patients.

What is the role of NFE2L3 in stem cell differentiation? Although several reports have identified *Nfe2l3* as a stemness marker gene, because of its early upregulation during stem cell differentiation, its precise role is still unresolved specifically because research on stem cells is as complex as it is fascinating and hopeful. Further efforts need to be devoted to this important area of research.

Finally, identification of the bona fide target genes of NFE2L3 is essential for a full understanding of its functions, its regulation, and to find the pathways regulated by NFE2L3. Although several potential target genes of NFE2L3 have been reported in the literature including *Prdx6* [79, 110], *Nqo1* [79, 87], *SMαA* [79], *SM22α* [79], *NOX4* [79], *Pparγ 2* [78], and *Nfe2l2* [78], no clear evidence has been provided for the in vivo relevance of these data.

Undoubtedly, the most exciting time for research on NFE2L3 is yet to come. There are still many aspects of its regulation and function that need to be better understood, including the control of its subcellular regulation, the range of target genes as well as its precise role in tumorigenesis. NFE2L3 has been the Cinderella of the CNC factors for many years; it is now time for this transcription regulator to come out into the spotlight.

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