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Regulation of the hypertonic stress response and other cellular functions by the Rel-like transcription factor NFAT5

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ABSTRACT

Stress, be it from environmental factors or intrinsic to the cell as result of growth and metabolism can be harmful to cells. Mammalian cells have developed numerous mechanisms to respond to diverse forms of stress. These mechanisms combine signaling cascades and activation of gene expression programs to orchestrate an adaptive response that will allow the cell to survive and resume its normal functioning. In this review we will focus on the transcription factor NFAT5, a fundamental regulator of the response to osmotic stress in mammalian cells. Identified in 1999, NFAT5 is the latest addition to the Rel family, which comprises the NF-κB and NFATc proteins. Though in some of its structural and functional features NFAT5 is a hybrid between these two major groups of Rel proteins, it has unique characteristics that make it stand on its own as a third type of Rel transcription factor. Since its discovery, NFAT5 has been studied mostly in the context of the hypertonicity stress response. The advent of mouse models deficient in NFAT5 and other recent advances have confirmed a fundamental osmoprotective role for this factor in mammals, but also revealed features that suggest it may have a wider range of functions.

1. Introduction

The transcription factor NFAT5 belongs to the Rel family, which comprises the NF-κB and NFATc proteins (Fig. 1). NFAT5 is the largest Rel protein of vertebrates with almost 1500 amino acids and a long transactivation domain of more than 900 amino acids [1,2]. While the DNA binding domain of NFAT5 is thought to have evolved from NF-κB, and indeed has structural and functional features found in both NF-κB and NFATc, the rest of the protein differs considerably from other Rel transcription factors. The best characterized NFAT5 function is its activation by hypertonic stress, in response to which it induces an osmoprotective gene expression program and the synthesis of some inflammatory cytokines. However, NFAT5 can also be induced by stimuli independent of osmotic stress, suggesting additional roles for this protein. The recent description of NFAT5-deficient animals has confirmed a major role of NFAT5 in the response to hypertonicity *in vivo*. In addition, this factor can regulate other processes in mammals, from embryonic development to cell migration. Here we will review the current knowledge and emerging questions on the function and regulation of NFAT5.

2. Relatedness of NFAT5 to other Rel proteins in vertebrates and other organisms

The Rel family of transcription factors (NF-κB and NFAT) is defined by a conserved DNA binding domain, the Rel domain (Fig. 2, also see review by Hogan et al. [3] for an excellent

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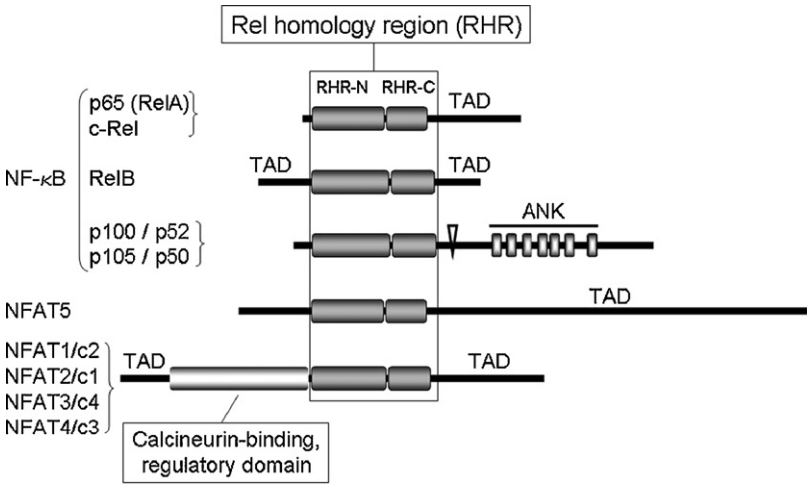

Fig. 1 – Schematic diagram of mammalian Rel proteins. Rel proteins share a structurally conserved Rel homology region (RHR). Their DNA binding loop is in the RHR-N domain and a dimerization domain is located in the RHR-C domain. The NF-κB family comprises five members. p65 (Rel A) and c-Rel have 551 and 619 amino acids, respectively, have short N-terminal regions and their transactivation domain (TAD) is located in their C-terminal region. Rel B has 557 amino acids and transactivation domains located at both its N- and C-terminal domains. p100/p52 and p105/p50 have 898 and 969 amino acids, respectively, and contain ankyrin repeats in their C-terminal domain (ANK). Their active forms, p52 and p50, are produced by proteolytic cleavage (indicated by an arrowhead). NF-κB proteins dimerize through their RHR-C domain, bind DNA as dimers and can combine to form different types of homo and heterodimers [52]. NFAT5 has 1455 amino acids and a large C-terminal region that harbors a hypertonicity-sensitive TAD. NFAT5 is homodimeric, with a dimerization interface in its RHR-C domain highly similar to that of NF-κB proteins. NFATc proteins are all activated by calcineurin and have a conserved N-terminal domain that contains calcineurin docking sites and the clusters of regulatory serines that control nuclear localization and transcriptional activity. The C-terminal regions of NFATc proteins differ considerably from each other and from NFAT5.

summary on the structure of different Rel proteins). These proteins have a long evolutionary history, being found from arthropods to mammals, including humans, while absent in nematodes and unicellular eukaryotes. Insects have two types of Rel proteins, Rel (NF-κB) and dNFAT, an ancient NFAT protein that was first detected by hybridization with a human NFAT5 DNA probe [4], and later identified in a functional screen in *Drosophila* [5]. dNFAT has been found in different species of insects and its Rel domain resembles mammalian NFAT5 more than any of the calcineurin-regulated NFATc, as it has a 51% amino acid identity to NFAT5 DNA binding domain and conserves its characteristic dimerization residues (Fig. 2). Other features shared by dNFAT and NFAT5 are that both are large (more than 1400 amino acids), lack the characteristic calcineurin docking sites and regulatory phosphoserine clusters of NFATc, and contain glutamine repeats. However, as they lack recognizable sequence similarities outside their DNA binding domains, it is currently unknown whether dNFAT is the functional equivalent to mammalian NFAT5, or instead it represents a unique protein, without a mammalian counterpart.

The DNA binding domain (DBD) of NFAT5 reflects a hybrid nature between the two other groups of Rel proteins, the calcineurin-regulated NFATc proteins and the NF-κB proteins [2,6–8]. It binds NFAT-like DNA elements, similar to those recognized by NFATc proteins [2], and although it has lower affinity for DNA than the DBD of NFATc, it has the ability to encircle the DNA, which might provide greater kinetic stability [7]. Compared to NFAT1/NFATc2, NFAT5 DBD has a stricter sequence requirement in its target DNA site, TGGAAAC/A/T, whereas NFAT1 can bind a wider range of variations around a T/A/CGGAA/C core motif [2] [7]. On the other hand, the NFAT5 DBD is a constitutive dimer and has a dimerization surface in its C-terminal half very similar to that of NF-κB proteins. This surface, together with and additional dimer interface (E′F loop) in the N-terminal half of the DBD, allows NFAT5 to encircle the DNA [7]. Like for NF-κB proteins, dimerization is an obligate requirement for NFAT5 to bind to DNA [6]. However, while the DNA binding site for NF-κB has to be symmetrical in that both halves of the dimer must contact a GG dinucleotide in opposite DNA strands, in the case of NFAT5 only one half of the dimer contacts the TGGAAA sequence whereas the other monomer can bind a non-consensus sequence [7]. Despite the homology between the NFAT5 and NF-κB dimerization domains over-expression of the NFAT5 dimerization domain in cells acted as a dominant negative selectively for NFAT5 without affecting the transcriptional activity of NF-κB nor NFATc proteins, suggesting that NFAT5 does not generally dimerize with other Rel proteins [6].

Apart from the homology in the Rel domain, there is no recognizable similarity outside this region between NFAT5 and NFATc or NF-κB proteins. Mammals have a single NFAT5 gene and three isoforms (NFAT5a, b and c) that differ in their first 76 amino acids in the N-terminal region [4]. NFAT5 lacks the conserved calcineurin docking sites and target phosphorylation residues present in the N-terminal regulatory region of

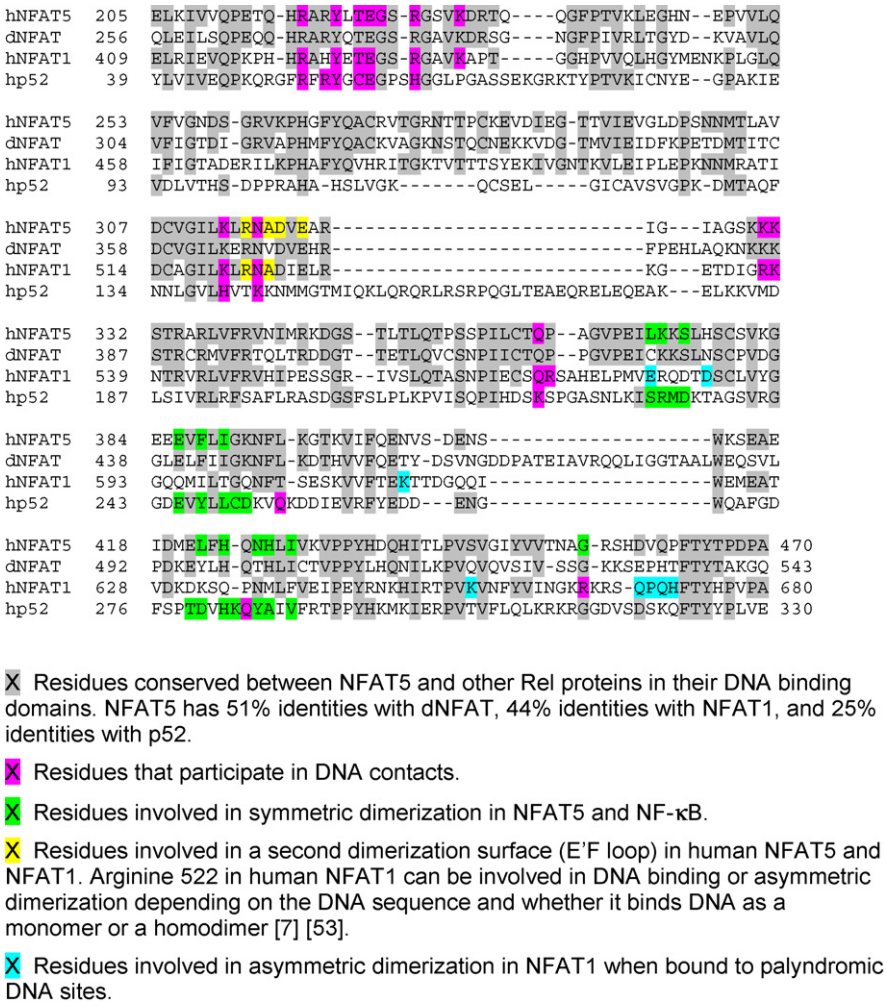

Fig. 2 – Alignment of the DNA binding domains of human NFAT5 (isoform NFAT5a) with *Drosophila* NFAT (dNFAT), human NFAT1 and human p52. Accession numbers are AAD38360 for NFAT5, AAG28468 for dNFAT, AAC50887 for NFAT1, Q00653 for human p100/p52. Colored residues are from structural analysis reported in Refs. [7,53]. Sequence alignment of NFAT5 with NFAT1 and p52 is the same described in Stroud et al. [7]. Sequence alignment of NFAT5 with dNFAT was done with basic local alignment search tool (BLAST), bl2seq, http://www.ncbi.nlm.nih.gov/BLAST/.

NFATc proteins (NFAT1-4). On the other hand, no mechanism similar to the cytoplasmic retention of NF-κB by IκB has being described for NFAT5.

The C-terminal domain of NFAT5 seems to be an acquisition of vertebrates as it can only be recognized in all the sequenced genomes of vertebrates but not in the insect protein dNFAT. This large NFAT5 region (almost 1000 amino acids) has been shown to contain the transactivation domain of NFAT5, which is activated and phosphorylated in response to hypertonicity [6,9].

3. Regulation of NFAT5 by hypertonicity: mechanisms and signaling pathways

Activation of NFAT5 involves three main processes: nuclear translocation, upregulation of its transcriptional activity, and increased NFAT5 synthesis. Of these, transcriptional activation has been the most extensively studied. Although it is likely that full, sustained activity of NFAT5 in response to hypertonicity activity requires the coordination of these three mechanisms, there are no available studies yet quantitating their relative contribution in different cell types.

3.1. *Nuclear translocation of NFAT5*

In contrast to NF-κB and NFATc proteins, which are retained in the cytoplasm in unstimulated cells, a substantial proportion of NFAT5, or even most of it, is constitutively nuclear in many cell types [2]. Exposure of cells to hypertonic conditions causes its complete nuclear translocation [10,6]. A recent study has identified three motifs in the N-terminal domain of NFAT5 that control its nuclear import and export: a nuclear localization sequence (NLS) (residues RKSRKRNPKQRPGVKRRD, in which the underlined RKR are essential for nuclear translocation), and an auxiliary export domain (AED) located N-terminal of the NLS that is required for nuclear export under hypotonic conditions independently of Crm1. In addition, one of the three NFAT5

Table 1 – Summary of studies on the regulation of NFAT5 by hypertonicity in different cell types

Function	Pathway/inhibitors tested	Cell type	References
Nuclear translocation	Insensitive to proteasome inhibitor MG-132.	MDCK, COS-7	[12,13]
	Partially inhibited by proteasome inhibitor MG-132.	HepG2	[13]
	PI3K inhibitor wortmannin (20 μM) inhibited translocation. Overexpression of ATM enhances translocation.	COS-7	[13]
	PI3K inhibitor wortmannin (20 μM) enhanced translocation.	AT cells (ATM deficient)	[13]
Transcriptional activitation	Inhibited by proteasome inhibitor MG-132.	MDCK	[12]
	Inhibited by p38 inhibitor SB203580 and p38 dominant negative.	NIH3T3	[14]
	Inhibited by Fyn dominant negative.	NIH3T3	[14]
	Reduced activity in $Fyn^{-/-}$ cells.	Mouse embryo fibroblasts	[14]
	NFAT5 is phosphorylated in Ser and Tyr.	MDCK	[10]
	SB203580 and PP2 (Src inhibitor) do not inhibit phosphorylation of NFAT5 in response to hypertonicity.	MDCK	[10]
	Overexpressed PKA catalytic subunit activates NFAT5, and PKA interacts with it.	HepG2	[15]
	Inhibited by wortmannin (1–20 μM).	HEK293, AT cells	[17]
	Overexpressed ATM enhances NFAT5 activity. ATM interacts with NFAT5.	HEK293	[17]
	Inhibited by dominant negative p85 or p110α siRNA.	HEK293	[18]

isoforms (NFAT5c) contains a Crm1-interacting, leucine-rich nuclear export signal (NES) (MPSDFISLLSADLDLESPK). It appears that hypertonicity inactivates the NES and AED, allowing full translocation of NFAT5 [11]. In this regard, it is to be expected that tonicity-responsive signaling pathways will regulate the masking or unmasking of these motifs, although specific mechanisms controlling the nucleocytoplasmic shuttling of NFAT5 are largely uncharacterized. Reports on the effect of drugs such as the proteasome inhibitor MG-132 and wortmannin have shown apparently contradicting results, with nuclear accumulation of NFAT5 being inhibited in one cell type but not in others [12,13] (see Table 1 for a summary of the effect of modifiers of several signaling pathways on NFAT5 activation in different cell types).

3.2. *Transcriptional activation of NFAT5 in response to hypertonicity*

NFAT5 has a large hypertonicity-responsive transactivation domain in its C-terminal region and a short, isoform-specific, N-terminal transactivation domain not activated by hypertonicity [6,9]. Analysis of the transcriptional activity of NFAT5 mutants and fragments of its C-terminal region fused to the heterologous GAL4 DBD, have shown that this region comprises four domains. Two of them display transcriptional activity inducible by hypertonicity and the other two act as modulators that enhance the activity of the former. Maximal transcriptional activity of NFAT5 in response to hypertonicity results from the synergistic effect of all four domains. Although NFAT5 is phosphorylated in response to hypertonic stress, phosphorylation correlated with increased transactivation activity only in some of the C-terminal subdomains, whereas others can be activated by hypertonicity without being phosphorylated [9].

Several kinases have been shown to have an effect on the transcriptional activity of this factor (Table 1). The p38 inhibitor SB203580 and a dominant negative p38 construct inhibited the hypertonicity-induced activation of NFAT5 in NIH3T3 cells [14]. In the same study, a dominant negative mutant of the Src family tyrosine kinase Fyn also suppressed NFAT5 activation, and $Fyn^{-/-}$ fibroblasts showed a weaker activation of this factor than wild-type cells. Inhibition of p38 in $Fyn^{-/-}$ fibroblasts caused a considerable downregulation of NFAT5 activity. These results, and the observation that hypertonicity induces phosphorylation of tyrosine and serine residues in NFAT5 [10], suggested that both kinases might be important for its transcriptional function and that full activation of NFAT5 could require the combined action of p38 and Fyn. However, neither SB203580 nor the Src kinase inhibitor PP2 affected the phosphorylatyion of NFAT5 induced by hypertonicity in MDCK cells [10]. Unfortunately, as both studies used different cell types and stimulation times, the question remains of whether the effect of p38 and Fyn involves the direct phosphorylation of NFAT5.

In addition to p38 and Fyn, PKA, ATM and PI3K can regulate NFAT5. Overexpression of the catalytic subunit of PKA (PKAc) in HepG2 cells activated NFAT5 in unstimulated cells [15]. In the same study, PKA and NFAT5 were coimmunoprecipitated in both unstimulated and osmotically stressed cells [15]. With regard to ATM, the PI3K inhibitor wortmannin was shown to inhibit NFAT5 activation. Wortmannin inhibits conventional PI3K at submicromolar concentrations but also inhibits the PI3-kinase-related kinases ATM and DNA-PKc when used at concentrations in the low micromolar range [16]. In HEK293 cells, 1 μM wortmannin caused a 50% inhibition of hypertonicity-induced activation of NFAT5 and 20 μM wortmannin reduced it by 70% [17]. The same study showed that overexpression of ATM, but not an inactive kinase mutant, enhanced the activity of NFAT5. In addition, hypertonicity activated ATM and this kinase coimmunoprecipitated with NFAT5. As with the PKA–NFAT5 association, the ATM–NFAT5 interaction was constitutive and not enhanced by hypertonicity, suggesting that NFAT5 might be already bound to one or several of its activating kinases in unstimulated cells. As

NFAT5 contains several potential ATM and DNA-PKc phosphorylation sites in its C-terminal region, mutational analysis was conducted to address their relevance in NFAT5 activation. This analysis, however, was conclusive only in part as the mutants overexpressed in HEK293 cells displayed reduced activity only in unstimulated cells but not in hypertonically treated cells [17]. A recent article has shown that PI3K itself is important in both the activation of ATM and the increase in NFAT5 activity by hypertonicity [18], since a dominant negative p85 or a p110α-specific siRNA inhibited the phosphorylation of ATM at S1981 and downregulated the activation of NFAT5-dependent reporters. Although this work indicates that a PI3K-ATM cascade is involved in activating NFAT5, the same laboratory had already shown that ATM is dispensable for NFAT5, as this factor was activated by hypertonicity and inhibited by wortmannin in AT cells, that lack ATM [17,13]. The sensitivity of NFAT5 to wortmannin in AT cells might suggest perhaps a redundant role of other PIKK, such as DNA-PKc, in the activation of NFAT5. However, results based on this inhibitor must be interpreted cautiously as this drug is known to inhibit at least two more kinases, SmMLCK [16] and mTOR [19], and might inhibit others, either directly or indirectly. Conclusive proof of the role of these kinases in NFAT5 activation, acting individually or coordinatedly regulating this factor, could be obtained by either using null mutants of them or suppressing their expression by RNA interference strategies. In addition, much is to be known about the mechanisms by which NFAT5 is activated by those kinases that have been shown to have an effect on it, and the actual phosphorylation sites that control its activity.

Different studies suggest that NFAT5 activation by hypertonicity requires a combination of multiple kinases, as the inhibition of either of them causes a substantial reduction of NFAT5 activity. These observations raise a number of relevant issues. First, must these kinases phosphorylate NFAT5 directly or do they act indirectly, via a sequential signaling cascade or even acting on other potential regulators of NFAT5? Second, is there any degree of redundancy between these kinases in the activation of NFAT5 or do they play specific functions in its activation, such as adjusting the amount of NFAT5 activity to the duration and magnitude of the stress? As mentioned above, the C-terminal transactivation domain of NFAT5 contains different regions, some of which are phosphorylated under hypertonic conditions whereas others respond to hypertonicity without apparent increases in their net amount of phosphorylation, suggesting the existence of different layers of regulation in its activity.

3.3. *Regulation of NFAT5 by increased synthesis*

In addition to the regulation of its nuclear accumulation and transcriptional activity, NFAT5 is also regulated via synthesis. Hypertonicity induces a substantial increase in the amount of mature NFAT5 protein [6,10]. The magnitude of the increase in NFAT5 expression suggests that this mechanism plays a significant role on its activity. In this regard, overexpression of NFAT5 in HEK293 cells can activate NFAT5-dependent reporters in the absence of hypertonic stimulation [9,17]. However, as the amount of ectopic NFAT5 in transfected cells can be much greater than the endogenous protein, these results are no formal proof that enhanced expression alone would suffice to activate NFAT5 target genes under physiological conditions. Induction of NFAT5 by hypertonicity is in part mediated by the stabilization of preexisting mRNA [20]. Stabilization is mediated by the 5′UTR, whereas the 3′UTR region of NFAT5 mRNA has a destabilizing effect under hypertonic conditions. Overall, the contribution of these sequences accounted for 30% of the increase in the amount of NFAT5 mRNA induced by hypertonicity in mIMCD3 cells derived from the inner renal medulla [20]. Other than this, the mechanism and regulators involved in NFAT5 mRNA stabilization by hypertonicity are unknown. On the other hand, no detailed studies are available either about regulatory regions acting as promoters or enhancers in the NFAT5 gene. It is expected that future studies will address these important questions and identify regulatory regions in the NFAT5 gene, as well as relevant signaling pathways controlling its expression.

4. Functions of NFAT5 in the hypertonic response

Initial observations with diverse dominant negative NFAT5 constructs and RNA interference [1,6,21,22], followed by the recent description and analysis of NFAT5-deficient mice [23,24] have shown that this factor is necessary for cells to mount an osmoprotective gene expression program in response to hypertonicity. These genes comprise at least the following ones: aldose reductase (AR), Na^+/Cl^--coupled betaine/γ-aminobutyric acid transporter (BGT1), Na^+-dependent myo-inositol transporter (SMIT), Na^+ and Cl^--dependent taurine transporter (TauT), *hsp 70.1*, and UT-A urea transporter (reviewed in Ref. [25]).

Mice lacking NFAT5 have severe renal defects associated to massive loss of cells in the renal medulla, which fail to express AR, SMIT and BGT1 in their naturally hypertonic environment and undergo apoptosis [23]. Similar viability defects have been found in the eye lens in transgenic mice that overexpress a dominant negative NFAT5 mutant in these cells [26], and inability to cope with a local hypertonic environment in lymphoid organs has also been reported as a possible cause for T and B cell dysfunction in NFAT5-deficient animals [24]. In addition, NFAT5 has been shown to be activated in neurons under conditions of experimental dehydration in rodents *in vivo* [27]. As shown by the Kultz and Burg laboratories, hypertonicity can result in genotoxic stress, causing DNA damage in the form of double strand breaks, that might lead to growth arrest and cell death [28–32]. By restoring the intracellular osmotic balance under hypertonic conditions, NFAT5 plays a prominent role in the ability of different organs and tissues to withstand osmotic stress.

NFAT5 also regulates the induction of some inflammatory cytokines under hypertonic conditions. When peripheral blood mononuclear cells are exposed to osmotic stress they can express different cytokines, such as IL-8, IL-1 and TNFα [33]. Inhibition of NFAT5 by overexpression of a dominant negative construct (NFAT5 DNA binding domain) downregulated the expression of lymphotoxin-β induced by hypertonicity and the induction of TNFα in Jurkat T cells

stimulated with the combination of phorbol ester and hypertonicity [6]. Similarly, activation of the TNFα promoter by hypertonicity in L929 cells was blocked by the same inhibitor or by an NFAT5-specific shRNA [22]. Chromatin immunoprecipitation experiments confirmed that NFAT5 bound to the TNFα promoter under hypertonic conditions [6]. Footprinting analysis of this promoter showed that two of the six NFAT sites are bound by NFAT5 and their mutation inhibits the activation of the TNFα promoter by hypertonicity [22].

The biological meaning of TNFα induction by hypertonicity deserves some consideration as this cytokine can have pro- and anti-apoptotic effects, depending on other environmental cues and the activity of intracellular pathways, particularly NF-κB and JNK [34,35]. TNFα is a major activator of NF-κB, and, relevant to the osmotic stress response, it can induce the expression of aldose reductase, an osmoprotective gene, in an NF-κB-dependent manner in human liver cells [36]. However, TNFα induced apoptosis in HeLa cells when combined with osmotic stress, despite increased NF-κB activation [37]. NF-κB can be activated by osmotic stress and induce the expression of COX2 [38] and adenosine A1 receptor (A1AR) [39], which are considered to have an osmoprotective function. In this context, it will be important to characterize the NFAT5 target gene signature and compare it with that of other stress-activated factors.

Besides its specific niche in the hypertonic stress response, it is unclear yet whether NFAT5 participates in cellular responses to a wider range of stresses. Kinases shown to upregulate NFAT5 transcriptional function such as p38 or ATM are not restricted to hypertonicity and can be activated by other stimuli [40–42]. Particularly, ATM is activated by DNA damage, hypertonicity and hypotonic shock. While it is not known whether genotoxic agents activate NFAT5, hypotonic shock inhibits it, causing its nuclear export [43]. As NFAT5 appears to require the concerted action of several kinases, it might be possible that stimuli that fail to activate the appropriate combination of kinases would have a much weaker effect on NFAT5 or alternatively, regulate it towards as yet unrecognized functions.

5. Other functions of NFAT5

Several lines of evidence indicate that NFAT5 in involved in other processes independent of, or at least not overtly connected to, the hypertonic response, such as embryonic development, integrin-induced cellular migration, and proliferation.

Mice lacking NFAT5 have dramatically reduced embryonic viability between days E13.5 and E17.5, and substantial perinatal lethality [23,24]. Only 50% of the NFAT5$^{-/-}$ embryos are present at day E17.5 and most of the animals that are born die around day 10. The abrupt drop in survival of NFAT5-mutant mice early after birth correlates with the progressive development of urinary concentrating ability, suggesting renal failure as the likely cause of post-natal lethality. However, the substantial rate of embryonic lethality might not owe to a renal defect, since maintenance of the extracellular milieu of the fetus is dependent on the placenta, not the fetal kidney. In this regard, Pax-2 knockout mice cannot develop kidneys but do not suffer embryonic lethality [44]. Although NFAT5 has been shown to be present in most developing organs in the mouse embryo [45], at present it is unknown what its developmental function might be.

Clustering of the integrin α6/β4 increases the expression of NFAT5 and stimulates its transcriptional activity in breast carcinoma cell lines [46]. Inhibition of NFAT5 impaired migration of carcinoma cells induced by integrin ligands without affecting their invasion capacity. Overexpression of this factor in colon and breast cancer cell lines enhanced their migration [46]. This integrin also activates NF-κB [47] and NFAT1, which controls both migration and invasion [46], in part by inducing autotaxin/ENPP2 [48]. In contrast, NFAT5 does not appear to regulate autotaxin [48]. The signaling pathways activating both factors have not been elucidated, although calcineurin might be involved in the α6/β4-mediated activation of NFAT1, since this is inhibited by cyclosporin A [48]. Still, much is to be known about the regulation of these factors and their target genes in cell migration and invasion. Such knowledge might uncover important aspects of tumor invasiveness.

Besides integrins, NFAT5 is substantially induced by stimulation of T lymphocytes with mitogens or via their T cell receptor (TCR) [6,21]. TCR-mediated induction of NFAT5 differs from hypertonicity in that the former requires calcineurin whereas the latter does not [21]. Calcineurin is a major controller of NFATc proteins and is fundamental in lymphocyte activation [3]. The upregulation of NFAT5 via TCR and calcineurin suggests that this factor might participate in T cell activation, although its function is still unknown. Mutant lymphocytes with only 25% of NFAT5 activity proliferated as well as wild-type cells in response to TCR stimulation, while on the other hand, they were much less resistant to hypertonic stress [24]. This result would not rule out a hypertonicity-independent role of NFAT5 in T cell function as it is possible that a reduced complement of NFAT5 might suffice during T cell activation.

Although the mRNA for NFAT5 can be detected in most tissues in the mouse, its protein is most abundant in cells with a higher proliferation rate *in vivo*, and many laboratory cell lines tested for NFAT5 have greater amounts of NFAT5 than quiescent cells [2]. With the exception of some neurons that can express relatively high levels of this factor, the largest levels of NFAT5 protein are found *in vivo* in the thymus and testis [21] and its expression is substantially enhanced in T lymphocytes activated with mitogens or via TCR [6,21]. These observations might suggest that NFAT5 could participate in the regulation of cellular proliferation under isotonic conditions. In this context, the question has been raised as to whether NFAT5 responsiveness to osmotic stress might imply an ability to respond to other stimuli that cause changes in cell volume or intracellular water availability, as such processes are relevant for cell growth and proliferation [49]. These notions are supported by (1) the observation that fibroblasts cultured in isotonic medium lacking free amino acids underwent a reduction in cell volume and increased the amount of nuclear NFAT5 [50], and (2) the interpretation that the greater expression of NFAT5 in proliferating cells might result from a reduction of available water volume as result of macromolecular crowding caused by a rapid biosynthetic rate [49]. The

former report is consistent with the finding that T cells expressing a dominant negative NFAT5 displayed poorer proliferation capacity than wild-type cells in amino acid-depleted medium [51]. Although the idea of a wider role for NFAT5 in cell proliferation is appealing, it is in apparent contrast with the observation that T cells with a 75% reduction in NFAT5 activity have no apparent proliferation defect when activated via TCR in isotonic medium [24], and mouse embryonic fibroblasts that lack NFAT5 show no overt defect either. In both cell types, however, lack of NFAT5 caused severe proliferative arrest and loss of viability under hypertonic conditions [24]. Even as it seems counterintuitive that proliferating and metabolically active cells upregulate NFAT5 for no reason, why they do so, and what role plays NFAT5 in these processes has yet to be answered.

6. Conclusion

Encoded by a single gene in mammalians, NFAT5 is the latest addition to the Rel family of transcription factors that already comprised several NF-κB and NFATc members. That NFAT5 is important for mammalians is evident from the severe phenotype of NFAT5-deficient mice. Although a fundamental role of NFAT5 in maintaining the intracellular osmotic balance and cell survival under hypertonic stress has been well established *in vivo* and *in vitro*, important gaps remain regarding the identity of its regulators and interactors, the mechanisms that control its expression and activation, and its postranlational modifications in response to stimuli. In addition, emerging evidence indicates that this protein participates in diverse processes such as embryonic development, cellular migration, and proliferation. Of these, almost everything from signaling pathways to potential gene targets has yet to be investigated. It is to be expected that future studies in these areas will yield detailed knowledge on the function and regulation of this intriguing Rel protein and perhaps reveal links to diseases involving cellular stress.

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