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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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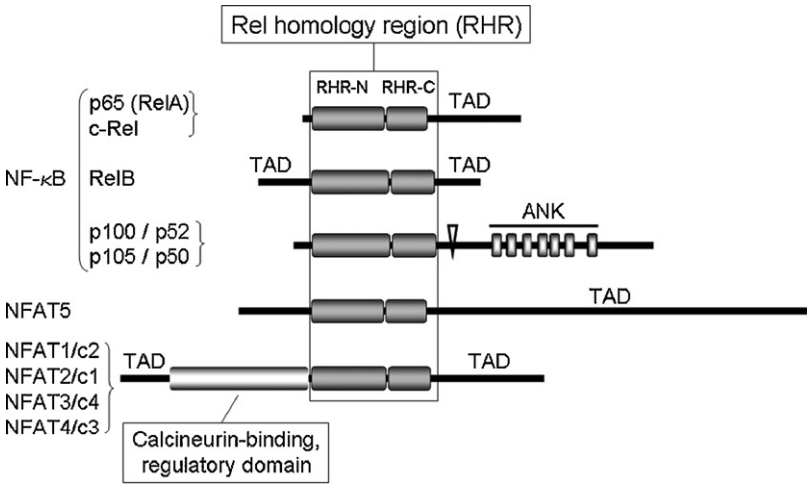

Fig. 1 – Schematic diagram of mammalian Rel proteins. Rel proteins share a structurally conserved Rel homology region (RHR). Their DNA binding loop is in the RHR-N domain and a dimerization domain is located in the RHR-C domain. The NF-κB family comprises five members. p65 (Rel A) and c-Rel have 551 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