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NFAT signaling in neural development and axon growth

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Abstract

The NFAT (nuclear factor of activated T-cells) family of transcription factors functions as integrators of multiple signaling pathways by binding to chromatin in combination with other transcription factors and coactivators to regulate genes central for many developmental systems. Recent experimental evidence has shown that the calcineurin/NFAT signaling pathway is important in axonal growth and guidance during vertebrate development. In fact, studies with triple NFATc2/c3/c4 mutant mice demonstrate that the extension and organization of sensory axon projection and commissural axon growth are both dependent upon NFAT activity. Neurotrophin and L-type calcium channel signaling modulate intracellular calcium levels to regulate the nuclear import and transcriptional activity of NFAT by activating the phosphatase calcineurin. The rephosphorylation and subsequent export of NFAT from the nucleus is mediated by several kinases, including GSK-3 β , which contribute to the fine tuning of NFAT transcriptional activity in neurons.

However, currently, no direct transcriptional targets for NFAT have been identified in a chromatin environment in the nervous system. Undiscovered are also the binding partners of NFAT that might combinatorially regulate specific genes important for neuronal development.

This review will discuss the current knowledge related to NFAT signaling in the nervous system development and the potential for future research directions.

Introduction

Transcription is a key regulation point for developmental processes as it allows for the integration of multiple signaling pathways.

Extensively characterized in the immune system, the NFAT family of transcription factors has now been shown to play a role in many vertebrate developmental systems, including the nervous system (for review see Graef, Chen et al. 2001; Crabtree and Olson 2002; Schulz and Yutzev 2004; Macian 2005).

NFAT functions as an integrator of multiple signaling pathways and achieves this through a combinatorial mechanism of transcriptional regulation. NFAT, along with other transcription factors and coactivators, integrates signaling pathways by binding to chromatin in a highly specific and concerted fashion only upon receiving the appropriate signaling cues. The composition of the NFAT transcription complexes assembled at the promoter and enhancer elements of target genes is thus dependent upon both signaling and chromatin context, which

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determines when and where NFAT complexes activate or repress transcription (Hogan, Chen et al. 2003; Im and Rao 2004).

In this review, we will focus on both the signaling pathways and transcriptional targets of NFAT relevant to the developing vertebrate nervous system.

The NFAT Family: overall signaling context

There are currently five different NFAT family members named NFATc1/2/c, NFATc2/1/p, NFATc3/4/x, NFATc4/3 and NFAT5/TonEBP (Rao, Luo et al. 1997; Graef, Chen et al. 2001; Macian 2005). All family members contain the rel DNA binding domain, however only NFATc1-c4 (here after referred to as NFAT unless specific members are cited) contains the Ca^{2+} sensor/translocation domain (Jain, Burgeon et al. 1995; Graef, Gastier et al. 2001). NFAT activation is dependent upon a rise in intracellular Ca^{2+} , which activates the serine/threonine phosphatase, calcineurin (Clipstone and Crabtree 1992; Jain, McCaffrey et al. 1993; Hogan, Chen et al. 2003). This phosphatase directly dephosphorylates several residues in the Ca^{2+} sensor/translocation domain of NFAT, resulting in exposure of a nuclear localization sequence and nuclear import of NFAT (Beals, Clipstone et al. 1997; Okamura, Garcia-Rodriguez et al. 2004).

Opposing this, the nuclear export of NFAT requires the sequential rephosphorylation of this domain by several kinases including GSK-3 β (Beals, Sheridan et al. 1997; Neal and Clipstone 2001; Sheridan, Heist et al. 2002).

Other post-translational modifications such as acetylation and sumoylation, as well as phosphorylation events distinct from those in the Ca^{2+} /translocation domain, also modulate NFAT transcriptional activity (Garcia-Rodriguez and Rao 1998; Yang, Davis et al. 2001; Terui, Saad et al. 2004).

An examination of the sequence homology within the NFAT family reveals that the evolutionary recombination event which joined the rel DNA binding domain with the Ca^{2+} sensor/translocation domain occurred near the origin of vertebrates, and lends support to the hypothesis that the Ca^{2+} /calcineurin/NFAT pathway plays a role in integrating newer vertebrate developmental pathways dependent upon Ca^{2+} signaling with evolutionarily older pathways (Graef, Gastier et al. 2001; Wu, Peisley et al. 2007).

There is accumulating evidence that the Ca^{2+} dependent calcineurin/NFAT signaling pathway is important in neuronal growth and guidance during vertebrate development (Graef, Mermelstein et al. 1999; Graef, Wang et al. 2003; Groth and Mermelstein 2003). The cooperative binding of NFAT with other transcription factors to form NFAT transcriptional complexes appropriate for neuronal development appears to be downstream of neurotrophin and netrin signaling pathways. In addition, modulation of Ca^{2+} levels through voltage gated Ca^{2+} channels might also allow NFAT transcription complexes to sense and integrate synaptic activity.

NFAT pathways in neural development

Calcineurin/NFAT signaling

Strong evidence that NFAT plays a role in vertebrate development came from mouse genetics studies. Targeted disruption of NFATc1 results in embryonic lethality with defects in cardiac valve formation (de la Pompa, Timmerman et al. 1998; Ranger, Grusby et al. 1998). Deletion of NFATc2 causes hyperproliferation of lymphocytes (Hodge, Ranger et al. 1996; Xanthoudakis, Viola et al. 1996), and also dysregulation of chondrogenesis (Ranger, Gerstenfeld et al. 2000). NFATc3 null mice have defects in myogenesis (Oukka, Ho et al.

1998; Kegley, Gephart et al. 2001), whereas null mice for NFATc4 appear to be developmentally normal. Interestingly, deletion of both NFATc4 and NFATc3 results in defects in vascular development and embryonic lethality at approximately E11.5 (Graef, Chen et al. 2001). As neuronal and vessel guidance share many of the same signaling pathways (Suchting, Bicknell et al. 2006), it was not surprising to find that 70% of these double mutant mice also show defects in sensory axon projection. Subsequent generation of triple NFATc2/c3/c4 mutant mice resulted in all animals having defects in sensory axon projection and commissural axon growth (Graef, Wang et al. 2003). These defects were specific to NFAT activation through the Ca^{2+} /calcineurin pathway, as mutant mice for calcineurin B also showed very similar defects in axonal outgrowth and die at E10 due to incomplete vascular development (Graef, Chen et al. 2001). In further support of a calcineurin/NFAT function in axonal outgrowth, the defects in both double NFATc3/c4 and triple NFATc2/c3/c4 mutant mice were recapitulated when embryos from wild-type pregnant mice were treated with cyclosporin A (CsA), a potent inhibitor of calcineurin (Graef, Chen et al. 2001; Graef, Wang et al. 2003).

Importantly, neuronal ganglia explants and neuronal culture experiments demonstrated that axonal outgrowth mediated by NFAT is dependent upon neurotrophin signaling. In fact, E10.5 trigeminal ganglia explants from wild-type mice cultured on collagen matrix physiologically respond to neurotrophins with axon outgrowth, but, strikingly, trigeminal ganglia explants taken from NFATc2/c3/c4 triple mutant or CsA treated embryos, showed little outgrowth in response to neurotrophins (Graef, Wang et al. 2003). Subsequent experiments comparing both wild-type and mutant explants clearly showed that the inability of triple NFAT mutant neurons to respond to neurotrophin signaling with robust axon outgrowth is due to their intrinsic loss of calcineurin/NFAT signaling; however this loss did not result in more cell death (Graef, Wang et al. 2003).

The implication is that NFAT transcriptional activity mediates axon growth and not the cell survival effects of neurotrophins signaling. However, in another study, NFATc4 promoted survival of granule neurons in the developing cerebellum (Benedito, Lehtinen et al. 2005). It is possible that different neuronal subtypes may use distinct NFAT transcriptional complexes to parse pro-survival and pro-growth signals.

Transcription reporter assays in cultured primary cortical and hippocampal neurons were also used to delineate some of the signaling components within the neurotrophin pathway important for NFAT activation. Cultured cortical neurons treated with BDNF stimulated NFAT-dependent transcription, and co-treatment with the calcineurin inhibitors CsA and FK506 blocked this activation (Graef, Wang et al. 2003). In fact, expression of EGFP-NFATc4 protein in cortical neurons showed nuclear localization and transcriptional activation upon BDNF treatment (Graef, Wang et al. 2003), which also enhanced endogenous NFATc4 transcription in cultured hippocampal pyramidal neurons (Groth and Mermelstein 2003). Addition of an inhibitor for phospholipase C or depletion of intracellular Ca^{2+} abolished the ability of BDNF to stimulate endogenous NFAT transcriptional activity in hippocampal neurons (Graef, Mermelstein et al. 1999; Groth and Mermelstein 2003).

In addition to BDNF, NGF plays a role in NFAT signaling in several neuronal populations, including cortical and hippocampal neurons. In fact, overexpression of the NGF-TrkA receptor in cortical neurons, promoted NFAT transcriptional activation in response to NGF stimulation. Interestingly, expression of TrkA receptor with mutations in the PLC γ 1-interaction site attenuated the ability of NGF to activate NFAT transcription (Graef, Wang et al. 2003). NGF has also been found to increase NFAT transcriptional activity in cultured spinal cells (Groth, Coicou et al. 2007).

Together, this shows that neurotrophin binding to Trk receptors appears to activate PLC signaling. This presumably results in cleavage of phosphatidylinositol 4,5-bisphosphate (PIP₂) and release of inositol 1,4,5-trisphosphate (IP₃), which causes an increase in intracellular Ca²⁺ through the IP₃-R1 receptor. Elevated Ca²⁺ levels then activates NFAT transcription via calcineurin (Figure 1). Moreover, neurotrophin signaling through NFAT appears to regulate expression of the IP₃-R1 receptor, as BDNF can drive a luciferase reporter containing the proximal IP₃-R1 promoter region (Groth and Mermelstein 2003), and one of four NFAT consensus site identified within the IP₃-R1 promoter was able to bind and shift NFAT from hippocampal nuclear extract or recombinant NFATc4 (Graef, Mermelstein et al. 1999). Interestingly, in cultured spinal cells, NFAT was able to activate COX-2 expression via BDNF signaling, but did not increase expression of IP₃-R1 receptor transcripts (Groth, Coicou et al. 2007). Perhaps distinct NFAT transcriptional complexes are used to activate different sets of genes during the developing and the adult nervous system.

An initial Ca²⁺ release, which stimulates NFAT-dependent transcription, can also be triggered by L-type channel or NMDA receptor. Their activation leads to an upregulation of IP₃-R1 through calcineurin in cerebellar granule neurons (Carafoli, Genazzani et al. 1999; Genazzani, Carafoli et al. 1999). NFAT transcriptional activity has been shown to be stimulated by an influx of Ca²⁺ through L-type channels in cultured hippocampal neurons (Graef, Mermelstein et al. 1999).

Therefore, a positive feedback loop might exist whereby an initial Ca²⁺ increase caused by neurotrophins signaling or L-type Ca²⁺ channels would activate NFAT transcriptional activity and feedback to modulate Ca²⁺ amplitude over time by the upregulation of more IP₃-R1 receptors (Figure 1). One possible hypothesis is that this feedback loop might be a mechanism to enhance Ca²⁺ signaling at the synapse if the upregulation of IP₃-R1 was coupled to its localization to the synapse (Graef, Chen et al. 2001). Interestingly, a recent study in zebrafish demonstrated that the calcineurin/NFAT signaling pathway regulates the morphological remodeling of axon terminals of olfactory sensory neurons (Yoshida and Mishina 2005). In fact, using GAP43-EGFP as a visual marker for axon terminal maturation, addition of CsA or VIVIT, an inhibitory peptide sequences derived from the calcineurin docking motif of NFAT, prevented axon terminal remodeling (Yoshida and Mishina 2005).

NFAT transcription complexes might also serve to integrate neuronal growth with guidance cues in the process of synaptogenesis. Netrins is a highly conserved family of guidance molecules that facilitate the development of neuronal circuits. These molecules provide attractive and repulsive guidance signals to direct neural and axonal pathfinding. The netrin-dependent guidance pathway seems to be relevant to NFAT transcriptional activity downstream of netrin/DCC signaling. Importantly, during the characterization of the triple mutant NFATc2/c3/c4 mice, it was noted that the defects in axon extension of commissural axons were similar to those of mutant mice for netrin-1 or its receptor DCC (Serafini, Colamarino et al. 1996; Fazeli, Dickinson et al. 1997). This observation was supported by the finding that in E13 rat dorsal spinal cord explants, netrin-1 stimulated axonal outgrowth, required calcineurin/NFAT signaling, and luciferase reporter assay in cultured cortical neurons showed that netrin-1 treatment could drive NFAT transcriptional activity through the DCC receptor (Graef, Wang et al. 2003).

An unanswered question is which set of transcription factors, along with NFAT, acts downstream of neurotrophin and netrin signaling to bind cooperatively to DNA and form active NFAT transcriptional complexes that would integrate axon growth with guidance cues.

Kinases/NFAT signaling

These positive signals through neurotrophins and netrin pathways that lead to activation of NFAT transcription are opposed by a less clearly defined set of protein kinases, which tend to promote NFAT nuclear export. NFAT is rapidly shuttled into and from the nucleus and this dynamic shuttling might be a mechanism to allow NFAT to sense intracellular oscillating Ca^{2+} levels (Hernandez-Ochoa, Contreras et al. 2007).

The relationship between NFAT and specifically active kinases within neurons has not been completely explored. Two negative regulators of NFAT activity have recently been identified and interestingly, both are located in the Down's syndrome critical region (DSCR) of chromosome 21 (Arron, Winslow et al. 2006; Gwack, Sharma et al. 2006). DSCR1 is a direct regulator of calcineurin and inhibits the calcineurin/NFAT pathway (Cano, Canellada et al. 2005; Arron, Winslow et al. 2006). It has also been shown to be a direct transcriptional target of NFAT (Cano, Canellada et al. 2005; Wu, Kao et al. 2007). Another negative regulator is DYRK1A, which is a serine/threonine kinase that has been shown to prime NFAT for additional phosphorylation by GSK-3 β (Gwack, Sharma et al. 2006). Therefore, an auto-feedback loop exists whereby both DSCR1 and DYRK1A affect the phosphorylation status and thus the transcriptional activity of NFAT (Figure 1).

GSK-3 β phosphorylation of NFAT has been reported in hippocampal neurons (Graef, Mermelstein et al. 1999), where it might synergize with DYRK1A to inhibit NFAT activity (Arron, Winslow et al. 2006). Modulation of this phosphatase/kinases loop is important in regulating the transcriptional activity of NFAT in neurons, and might be a way for neurons to specifically tune NFAT activity during axon outgrowth and pathfinding.

Conclusions and Perspectives

How does a developing organism achieve such cell-type diversity while only using a limiting set of signaling pathways and transcription factors? We can partially explain this diversity through a combinatorial mechanism of transcriptional regulation, whereby developmental signaling and chromatin context are integrated by different combinations of transcription factors to achieve activation of a diverse set of genes.

NFAT is an important player in the developing and likely in the adult nervous system. As calcineurin is highly expressed in the brain, and transgenic mice containing NFAT reporters show that the brain is the organ having the highest levels of NFAT transcriptional activity (Plyte, Boncristiano et al. 2001; Wilkins, Dai et al. 2004), it is important to identify key genes regulated by NFAT in the vertebrate nervous system.

However, currently no direct transcriptional targets for NFAT in the nervous system have been identified in a chromatin environment. In this regard, the use of chromatin immunoprecipitation (ChIP) combined with genome wide microarrays, the so called ChIP on-chip technology, may be warranted to identify NFAT target genes during neuronal development.

Missing is also the identification of binding partners of NFAT that might combinatorially regulate specific genes important for neuronal development. Since NFAT activity has also been shown to be stimulated by substance P (Seybold, Coicou et al. 2006), bradykinin (Jackson, Usachev et al. 2007), and Wnt (Saneyoshi, Kume et al. 2002), the choice of binding partners within this transcriptional dance is plentiful.

We can speculate that genes in developing neurons are regulated by integrating transcription factors, such as NFAT (Figure 1), that are dependent upon both modulation of Ca^{2+} levels within neurotrophin and guidance signaling pathways.

What those other factors are and at which promoters they function is still poorly understood, and it is worth further investigation.

Finally, we envision a potential role for NFAT in axon re-growth and regeneration following axonal injury. In fact, molecular mechanisms important for neuro-developmental axon growth are often re-activated following axonal injury in the adult during axon regeneration. Therefore, investigating the function of NFAT and its potential target genes following axon injury might unveil a novel role for NFAT in regenerative axon growth and provide novel pro-regeneration targets for molecular interventions.

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Figure 1. Shown is a representative scheme of signaling pathways integrated by NFAT within developing neurons.