NFAT: ubiquitous regulator of cell differentiation and adaptation

Valerie Horsley and Grace K. Pavlath

Department of Pharmacology, Emory University, Atlanta, GA 30322

The nuclear factor of activated T cells (NFAT) proteins are a family of transcription factors whose activation is controlled by calcineurin, a Ca^{2+}-dependent phosphatase. Originally identified in T cells as inducers of cytokine gene expression, NFAT proteins play varied roles in cells outside of the immune system. This review addresses the recent data implicating NFAT in the control of gene expression influencing the development and adaptation of numerous mammalian cell types.

The formation and function of tissues requires the acquisition of cellular phenotype during differentiation. Differentiation involves expression of genes that specify cellular fate and down-regulation of genes that hold cells in a pluripotent state. Once a tissue forms, changes in gene expression are also important as cells within a tissue adapt to environmental changes through a pattern of new protein expression. These cellular adaptations allow cells within a tissue to survive and maintain function. Thus, the control of gene expression is important for the formation, function, and maintenance of tissues.

Regulation of gene expression occurs through a variety of signaling pathways. Calcineurin is a Ca^{2+}/calmodulin-dependent protein phosphatase that is a downstream target of intracellular Ca^{2+} signaling (Rusnak and Mertz, 2000). The best-characterized substrate of calcineurin is the NFAT* family of transcription factors (NFATC1–C4) (Rao et al., 1997). During periods of sustained elevations of calcium, calcineurin dephosphorylates NFATC1–C4, allowing NFAT to translocate to the nucleus. This nuclear translocation is blocked by cyclosporine A (CSA), which blocks calcineurin activity. Once in the nucleus, NFAT binds to consensus DNA sites and controls gene transcription. Upon cessation of the Ca^{2+} signal, termination of NFAT signaling occurs through rephosphorylation of NFAT by kinases such as GSK-3 (Graef et al., 2001b), resulting in its translocation to the cytoplasm.

Initially, different NFAT family members were considered to have redundant functions but now are known to have distinct roles in cellular physiology. The varied expression profiles among cell types provided the first clue to functional nonredundancy (Rao et al., 1997). Further evidence was provided by activation of specific isoforms even when multiple isoforms are expressed within a cell. For example, in T cells and skeletal muscle cells, different NFAT isoforms translocate to the nucleus at specific stages of differentiation in response to a Ca^{2+} signal (Abbott et al., 1998; Adachi et al., 2000). The examples discussed below provide evidence for distinct functions of individual NFAT isoforms in the development and adaptation of a wide range of cell types (Tables 1 and 2).

NFAT regulates cell differentiation and development

NFAT signaling controls multiple steps in the development of the cardiovascular system. In the embryonic heart, NFATC1 is initially expressed broadly, but by E11.5 becomes restricted to cells that will eventually form the heart valves. NFATC1$^{-/-}$ mice have defective heart valve development and abnormalities in the cardiac septum (de la Pompa et al., 1998; Ranger et al., 1998). The signals that regulate NFATC1 expression and the downstream gene targets of NFATC1 clearly function to pattern heart organization during development.

NFAT also regulates peripheral vascular development during angiogenesis. Angiogenesis is a multistep process that involves cellular proliferation, differentiation, and migration of both endothelial cells and vascular smooth muscle cells (VSMCs) to give rise to mature blood vessels. During embryogenesis, NFATC3 and NFATC4 are expressed in perivascular tissues that direct the developing endothelial cells and VSMCs. NFATC3/C4 double null mice display disorganization of blood vessels and poor vessel wall integrity, leading to early embryonic lethality (Graef et al., 2001a). Dissection of the genes regulated by NFATC3 and NFATC4 will elucidate how tissue interactions occur to give rise to the peripheral vascular system.

NFAT signaling is also important in endothelial cells in response to vascular endothelial growth factor (VEGF).

Table I. **Evidence for the role of NFAT isoforms in the development of numerous cell types**

Tissue/cell type	NFAT isoform implicated	Methods used to determine role for NFAT	Target gene	Function	References
Heart	NFATC1	NFATC1$^{-/-}$ mice	?	Development of heart valve	de la Pompa et al., 1998; Ranger et al., 1998
Vascular endothelial cells (primary cells)	NFATC2	EMSA and reporter assays[1]; PG rescue of CSA effect in vitro/in vivo	COX2	VEGF signaling; PG synthesis during angiogenesis	Armesilla et al., 1999; Hernandez et al., 2001
Perivascular tissue	NFATC3/C4	NFATC3/C4 double null mice	?	Organization of developing blood vessels	Graef et al., 2001a
Skeletal muscle reserve cells	NFATC1	Overexpression and inhibition of NFAT in primary muscle cultures	myf-5	Expression of myf5	Friday and Pavlath, 2001
Skeletal muscle myotubes	NFATC2	NFATC2$^{-/-}$ mice	?	Skeletal muscle growth	Horsley et al., 2001
Skeletal muscle myoblasts	NFATC3	NFATC3$^{-/-}$ mice	?	Formation of primary myofibers	Kegley et al., 2001
Chondrocytes	NFATC2	NFATC2$^{-/-}$ mice	?	Inhibition of chondrogenesis	Ranger et al., 2000
Keratinocytes (primary cells)	NFATC3	Reporter assays[2]; NFAT inhibition; CSA treatment	p21$^{WAF1/CIP}$	Activation of p21 during differentiation	Santini et al., 2001
Adipocytes (cell line)	NFATC2/C4	EMSA and reporter assays[3]; CSA treatment	aP2	Expression of adipocyte specific genes during differentiation	Ho et al., 1998

PG, prostaglandin.
[1]COX2 promoter construct.
[2]NFAT reporter and p21 promoter constructs.
[3]aP2 promoter construct.

VEGF treatment results in translocation of NFATC2 to the nucleus of primary endothelial cells (Armesilla et al., 1999). Once in the nucleus, NFATC2 binds to the promoter of cyclooxygenase (COX)2, a key enzyme in prostaglandin synthesis, and activates transcription (Hernandez et al., 2001). The production of prostaglandins downstream of NFAT signaling is necessary for the migration of endothelial cells to allow the proper formation of endothelial tubes and angiogenesis to occur in vivo. This function of NFATC2 in endothelial cell migration appears to be postnatal since

NFATC2$^{-/-}$ mice are viable with no reported developmental defects in angiogenesis.

The musculoskeletal system is also dependent on NFAT signaling. Skeletal muscle tissue forms from the proliferation, differentiation, and fusion of muscle precursor cells to form multinucleated muscle cells. NFATC3$^{-/-}$ mice have embryonic defects in the formation of primary myofibers, the first multinucleated muscle cell (Kegley et al., 2001). NFATC2 plays a role in the postnatal growth of skeletal muscle. NFATC2$^{-/-}$ mice form small multinucleated mus-

Table II. **Evidence for the role of NFAT in cellular adaptation**

Cell type	NFAT isoform implicated	Methods used to determine role for NFAT	Target gene	Function	References
Pancreatic islet cells (cell line)	NFATC2	EMSA and reporter assays[1]	Glucagon	Expression of glucagon	Furstenau et al., 1999
Epidermal cell (cell line)	?	Reporter assays[2] in vitro and in vivo	?	Response to UV radiation	Huang et al., 2000
Cardiac muscle	NFATC4	Overexpression in transgenic mice	?	Cardiac hypertrophy	Molkentin et al., 1998
Skeletal myofibers (in vitro)	NFATC1	Nuclear localization	?	Response to nerve stimuli	Liu et al., 2001
Skeletal muscle cells (cell line)	?	Reporter assays[3]	MyHC II	Expression of MyHC II isoforms	Allen et al., 2001
VSMCs (primary cells)	NFATC1/C2	Reporter assays[4]	?	Agonist induced signaling	Boss et al., 1998
ISMCs (isolated tissue)	NFATC3	Nuclear localization; reporter assays[5]	?	Response to PDGF	Stevenson et al., 2001

EMSA, electrophoretic mobility shift assay.
[1]Glucagon promoter constructs.
[2]NFAT reporter construct; NFAT-luciferase transgenic mice.
[3]MyHC II promoter constructs.
[4]NFAT reporter construct.
[5]NFAT-luciferase transgenic mice.

cle cells due to a defect in the recruitment and/or fusion of myogenic cells with nascent multinucleated muscle cells (Horsley et al., 2001). In addition, NFAT may regulate a subpopulation of myogenic cells called reserve cells. These cells remain unfused in cultures of multinucleated muscle cells and express the muscle regulatory protein, myf-5. NFATC1 enhances the expression of myf-5 in these cells, suggesting that NFAT may regulate properties of reserve cells (Friday and Pavlath, 2001). Thus, NFAT has distinct functions in multiple steps of myogenesis.

Cartilage formation in the adult is critical for the movement of joints and is often disrupted in osteoarthritis. The differentiation of adult mesenchymal stem cells into cartilage forming cells is associated with NFATC2 up-regulation (Ranger et al., 2000). Older NFATC2$^{-/-}$ mice have impaired ambulation and decreased joint motion, resulting from excess cartilage formation due to increased chondrocyte cell proliferation and differentiation. In adult chondrocytes, NFATC2 acts as a negative regulator to promote the appropriate formation of cartilage within adult joints.

The structure and function of the epidermis depends on the balance between proliferation and differentiation of keratinocytes. Differentiation of primary keratinocyte cells in vitro is associated with nuclear localization of NFAT and blocked by CSA (Santini et al., 2001). As keratinocytes differentiate, cell cycle withdrawal is induced by expression of p21$^{WAF1/CIP1}$, an inhibitor of cyclin-dependent kinases. Activation of p21 expression is dependent on a calcineurin-induced physical association of NFAT with Sp1/Sp3. Thus, an NFAT-Sp1/Sp3 signaling pathway is required for keratinocyte differentiation in vitro. Further work is needed to determine whether NFAT regulates such differentiation in vivo.

NFAT proteins also appears to regulate adipocyte differentiation in vitro (Ho et al., 1998). During fat cell formation, a population of uncommitted mesodermal precursor cells are induced to express adipocyte specific genes such as fatty acid binding protein and aP2. A role for NFAT is suggested by the fact that NFATC2 and NFATC4 bind to the promoter of aP2 in differentiated 3T3-L1 adipocytes. Furthermore, CSA blocks adipocyte differentiation. More studies are needed to conclusively show that NFAT signaling regulates adipogenesis both in vitro and in vivo.

Role of NFAT in cell adaptation

Cellular adaptation in response to environmental changes allows tissue function and survival. NFAT has recently been implicated in the adaptation of numerous cell types in response to external stimuli from the environment (Table II).

Glucagon is an important regulator of blood glucose levels by stimulating either glycogenolysis or gluconeogenesis in the liver. Glucagon synthesis in pancreatic islet cell lines is regulated by multiple calcium pathways including NFAT (Furstenau et al., 1999). Upon membrane depolarization, NFATC2 translocates to the nucleus, binds to the glucagon promoter together with the transcription factor HNF-3β, and activates transcription. If these results are verified in vivo, NFAT signaling in pancreatic cells may be critical for blood glucose homeostasis.

Mammalian cells respond to UV radiation by activating signal transduction pathways in a process known as the "UV response." This UV response elicits a number of biological effects in skin to protect the epidermis from DNA damage. NFAT-mediated transcription is induced in epidermal cells in response to UV radiation in both epidermal cell lines and in skin in vivo (Huang et al., 2000). Thus, NFAT proteins likely regulate adaptation in the epidermis following exposure to UV radiation.

NFAT may also regulate the adaptive responses of all muscle cell types in the body. Hypertrophy of cardiac and skeletal muscle cells occurs in response to increased workload and various agonists. Cardiac specific expression of a constitutively active calcineurin or NFATC4 in transgenic mice is sufficient to induce cardiac hypertrophy (Molkentin et al., 1998). Whether calcineurin signaling is involved in all forms of cardiac hypertrophy is controversial (Leinwand, 2001). A role for calcineurin and NFAT in skeletal muscle hypertrophy is also not without controversy (Bodine et al., 2001; Rommel et al., 2001). CSA inhibits overload induced hypertrophy in skeletal muscle, suggesting a role for calcineurin, but whether NFAT is a downstream mediator is unknown (Dunn et al., 1999). IGF-1 induces muscle cell hypertrophy and nuclear translocation of NFATC1 in vitro (Musaro et al., 1999). Further work is required to determine if NFAT signaling is directly involved in cardiac and skeletal muscle hypertrophy.

In response to motor neuron activity, distinct patterns of gene expression are activated in skeletal muscle fibers, leading to slow and fast twitch myofibers. These subtypes are characterized by expression of different isoforms of myosin heavy chain (MyHC). A proposed role for calcineurin in slow myofiber gene expression (Olson and Williams, 2000; Serrano et al., 2001) has been debated (Calvo et al., 1999; Swoap et al., 2000). The studies by Liu et al. demonstrate that patterns of electrical stimulation characteristic of slow motor neurons induce nuclear translocation of NFATC1 in isolated myofibers (Liu et al., 2001). This evidence is the first link between activation of NFAT and a physiologic stimulus in myofibers. Recent data suggest that NFAT may regulate not only slow but fast myofiber phenotype. Calcineurin activation of the fast MyHC IIa promoter requires NFAT consensus sequences (Allen et al., 2001), but constitutively active NFAT down-regulates transcription of fast MyHC type IIb and IIx/d promoters. These data contribute to the complexity of calcineurin and NFAT signaling in skeletal muscle physiology.

Smooth muscle cells also utilize NFAT signaling for adaptation. In response to extracellular signals, smooth muscle cells alter gene expression, remodel their extracellular matrix, and begin cell proliferation and migration. NFAT proteins are expressed in VSMCs in culture (Boss et al., 1998), and in native ileal smooth muscle cells (ISMCs) (Stevenson et al., 2001). In response to platelet-derived growth factor (PDGF), NFAT-mediated transcription occurs in both ISMCs and VSMCs. Since PDGF is an important smooth muscle mitogen, these data suggest that activation of NFAT may be involved in smooth muscle cell adaptation. These examples illustrate that NFAT-regulated gene transcription may be a common signaling mechanism in cardiac, skeletal, and smooth muscle cells.

Future perspectives

The identification of NFAT in nonimmune cells has led to recent data implicating this family of transcription factors in the

development and adaptation of multiple cell types. Future challenges in the field include corroborating results obtained in cell culture to that in native tissues as well as determining whether NFAT regulates the expression of endogenous target genes or occupies endogenous promoters in vivo. Additionally, the upstream receptor–ligand interactions that lead to activation of NFAT are not well characterized in many cell types. Furthermore, many of the transcription factors that work in synergy with NFAT are unknown. Defining the upstream ligands and receptors, transcriptional partners and genes regulated by NFAT signal transduction pathways will elucidate how development and adaptation occurs in multiple cell types.

Manipulation of the cellular responses regulated by NFAT may be clinically relevant in nonimmune cells. The currently available drugs, such as CSA and FK506, both inhibit calcineurin activity and thus inhibit NFAT signaling indirectly. Manipulating NFAT pathways to treat disease will be facilitated by drugs that directly inhibit NFAT activation without affecting calcineurin activity toward other substrates. Such NFAT specific inhibitors would likely decrease the number of side effects associated with current therapies that inhibit calcineurin-dependent pathways. Utilization of upstream activators of NFAT signaling may also aid in disease treatment. Further study of the biology of NFAT signaling in a range of cell types will facilitate the manipulation of these pathways to treat disease.

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