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Review

Multiple roles of the *DSCR1 (Adapt78* or *RCAN1)* gene and its protein product Calcipressin 1 (or RCAN1) in disease

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Abstract. The *DSCR1 (Adapt78)* gene[1] is transiently induced by stresses to temporarily protect cells against further potentially lethal challenges. However, chronic expression of the *DSCR1 (Adapt78)* gene has now been implicated in several pathological conditions including Alzheimer's disease, Down syndrome and cardiac hypertrophy. Calcipressin 1 has been shown to function through direct binding and inhibition of the serine threonine protein phosphatase Calcineurin. Pharmacological inhibition of calcineurin, by the immunosuppressive drugs cyclosporin A and FK506, affects a wide variety of diseases. It is, therefore, likely that this endogenous calcineurin inhibitor, calcipressin 1, may also play a role in a variety of human diseases.

Key words. Down syndrome; Alzheimer's disease; cardiac hypertrophy; calcineurin; calcipressin 1; MCIP1; *DSCR1; Adapt78;* RCAN1.

The *DSCR1 (Adapt78)* or *RCAN1* gene

Structure and nomenclature

The Human *DSCR1 (Adapt78)* gene is located on chromosome 21 (fig. 1) in the region of 21q22.12 [1]. This gene is involved in cellular adaptation to oxidative stress, transiently protecting cells that have been primed with a low dose of an oxidant stress against subsequent, potentially lethal, challenges. It has been shown to be involved in Alzheimer's disease as well as the prevention of muscle hypertrophy and is likely to be involved in several disease pathways. Understanding *DSCR1 (Adapt78)* and how it functions could prove to have a great impact on human health and disease. This review touches on a few possibilities in this area.

The *DSCR1 (Adapt78)* gene was found by the Fuentes lab, as a gene thought to lie within the Down Syndrome Critical Region and named *DSCR1* [2]. It was later found to lie just outside of this region, and the name was modified to *Down Syndrome Candidate Region 1* gene [3]. It was also independently identified in our laboratory as a gene induced during cell adaptation to oxidative stress, and dubbed *Adapt78* [4, 5]. Initially the protein names used for this gene followed the gene names. When the protein was later discovered to inhibit calcineurin, it was named calcipressin 1 based on its function [6]. Other names based on function have also been suggested. Calcineurin binding protein (CBP1) was chosen by Gorlach et al., but this is already in use for the calcium binding protein [7]. In yeast, it was named Rcn1 [8], but this name

[1] Please note that the mammalian *DSCR1* gene is also called *Adapt78* or *RCAN1*, and its protein products have been named Calcipressin1, MCIP1 and RCAN1. A proposal to adopt a single gene name of *RCAN1* and a protein name RCAN1 (for Regulator of Calcineurin) has been endorsed by the HUGO Gene Nomenclature Committee, but final approval must await agreement from a majority of researchers in the field.

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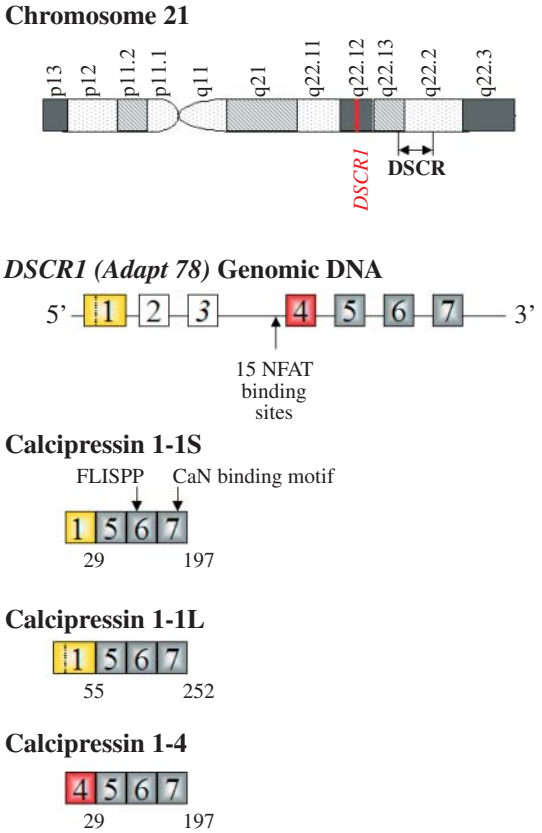

Figure 1. Chromosome 21: *DSCR1 (Adapt78)* is located on chromosome 21 in the region q22.12. This lies outside of the Down Syndrome Candidate Region (DSCR). DSCR1 (Adapt78) Genomic DNA: *DSCR1 (Adapt78)* consists of seven exons separated by six introns that are alternatively spliced and vary in their 5' exon, but all contain exons 5, 6 and 7. There is a cluster of 15 NFAT binding sites on the *DSCR1 (Adapt78)* gene, 5' to exon 4, which may function as an alternative promoter region for the exon 4 splice variant. Calcipressin 1 protein: All calcipressin 1 isoforms share 168 amino acids encoded by exons 5, 6 and 7, as well as the conserved FLISPP sequence found in all of the calcipressin family members. This motif shares homology with the serine poline (SP) boxes found in NFAT protein family members. All calcipressin 1 isoforms contain a putative calcineurin-binding motif (PKIIQT). The major isoforms of the protein that have been reported are calcipressin 1-1, with the N-terminal amino acids encoded by exon 1, and calcipressin 1-4, with the N-terminal amino acids encoded by exon 4. More recently, calcipressin 1-1L has been reported, with the N-terminal amino acids encoded by an elongated version of exon 1, which encodes 26 amino acids in addition to the 29 found in exon 1 of calcipressin 1-1.

has already been used for another protein [9]. It was also named myocyte-enriched calcineurin interacting protein 1 (MCIP1), and modified later to modulatory calcineurin interacting protein 1 to reflect its function [10, 11]. At the present time the most widely used terminology for this gene is *DSCR1 (Adapt78),* and the most widely used terminology for the protein is calcipressin 1; therefore, we have chosen this nomenclature for our review.

It now appears clear that the protein product of the mammalian *DSCR1* or *Adapt78* gene is a regulator of the calcineurin phosphatase. A proposal to adopt a single gene name of *RCAN1* and a protein name RCAN1 (for Regulator of Calcineurin 1) has been endorsed by the HUGO Gene Nomenclature Committee, but final approval must await agreement from a majority of researchers in the field.

The *DSCR1 (Adapt78)* gene consists of seven exons, which are alternatively spliced, separated by six introns. All messenger RNA (mRNA) isoforms that have been seen share exons five through seven, encoding 168 amino acids. Either exon 1 or exon 4 initiates the vast majority of the observed forms. The isoform containing exon 2 has been reported only in fetal liver and brain, and isoform 3 has not yet been detected [3]. The mRNA containing exon 1 encodes two potential translation initiation sites and might be translated into two proteins: calcipressin 1-1 short (calcipressin 1-1S), encoding a 197-amino acid protein, and calcipressin 1-1 long (calcipressin 1-1L) encoding a 252-amino acid protein. Isoform 4 encodes one protein of 197 amino acids. It has been suggested that isoform 4 may be initiated by an alternative promoter due to the distance from the promoter at the 5' end [10].

Expression (isoforms/tissues)

DSCR1 (Adapt78) gene expression is significant in several tissues, particularly in human brain, spinal cord, kidney, liver, mammary gland, placenta, skeletal muscle and heart [12]. In adults, *DSCR1 (Adapt78)* isoform 1 is most highly expressed in the heart, brain, muscle and pancreas, while isoform 4 is most highly expressed in heart, liver, muscle, placenta, pancreas and kidney [3]. In the brain it is expressed at the highest levels in the *thalamus*, *medulla oblongata*, cerebral cortex, *hippocampus* and *substantia nigra*, predominantly in neurons. In the human brain mRNA levels of isoform 1 are much higher in concentration than isoform 4 [12].

DSCR1 (Adapt78) is widely expressed in the central nervous system (CNS) during early embryonic development, later being restricted to regions with actively proliferating or differentiating neurons [13]. This corresponds to data from the *Drosophila* homologue that is upregulated in the head during development [14]. Expression in the liver and heart is much more homogeneous [2]. It is also upregulated during muscle differentiation and elevated in type I (slow oxidative) muscle [15]. This gene is not only inducible by oxidative insults, but is also highly inducible by calcium and agents that alter calcium distribution [5, 16, 17].

Family

Although the *DSCR1 (Adapt78)* family has not been found in bacteria, family members have been found in

most eukaryotic models including orthologues in *Saccharomyces cerevisiae, Caenorhabditis elegans, Aspergillus nidulans, Cryptococcus neoformans, D. melanogaster* and higher eukaryotes [2, 5–7, 15–17]. In humans there are three family members, including *DSCR1 (Adapt78)* on chromosome 21, *DSCR1 like 1* (*DSCR1-L1*, also known as *ZAKI-4*) on chromosome 6 and *DSCR1-L2* on chromosome 1. These sequences are highly conserved between mice and humans [18].

The DSCR1 (Adapt78) protein product calcipressin 1, MCIP1 or RCAN1

Structure

Calcipressin 1, the protein product of the *DSCR1 (Adapt78)* gene, has different isoforms as a result of the alternative mRNA splicing. Calcipressin 1 proteins vary in size from approximately 18 to 80 kDa [unpublished results from our laboratory]. Calcipressin 1 proteins include the family signature FLISPP motif – a serine proline (SP) box similar to those found in the nuclear factor of activated T cells (NFAT) family of proteins, and a putative nucleic acid binding domain [2, 15, 19]. At least two serine residues of these proteins can be phosphorylated, one by mitogen-activated protein kinase (MAPK) and one by glycogen synthase kinase 3 (GSK-3). Calcipressin 1 has been reported to be found both in the nucleus and cytoplasm. Its location may depend on whether or not calcipressin 1 is bound to calcineurin, since calcineurin has been reported to be located in the cytoplasm, moving to the nucleus when its autoinhibitory domain is displaced and the protein becomes active [15, 20, 21]. The last 33 amino acids in the carboxyl-terminal portion have been shown to play an important role in its nuclear localization. The C-terminal region (amino acids 115–197) is also responsible for binding to calcineurin and there is a SH2 ligand domain located in this region [20].

Function

Recently, it has been reported that the protein products of *DSCR1* (*Adapt78*), calcipressin 1s, are able to bind to and inhibit the catalytic subunit of calcineurin (protein phosphatase 2B) [6, 7, 15]. Since these proteins have been discovered to be endogenous inhibitors of calcineurin, they have been dubbed calcipressins [7]. It seems that the calcineurin binding domain is located in the C-terminal portion of calcipressins, which is present in all calcipressin isoforms. Therefore, all calcipressin isoforms should be able to inhibit calcineurin [15]. Phosphorylation of calcipressin 1 may alter its ability to inhibit calcineurin, although conflicting results have been reported: it has been shown to increase calcineurin inhibition [19]; decrease calcineurin inhibition [22]; or have no effect on calcineurin [11]. These conflicting results may be due to the levels of calcipressin 1, since the yeast homologue of calcipressin 1, regulator of calcineurin (RCN), can stimulate calcineurin signaling depending on its level [23]. Phosphorylation also increases the rate of calcineurin degradation, possibly via the proteasome [19].

Calcineurin is a calcium-dependent serine-threonine protein phosphatase under the control of calcium/calmodulin, which has several known substrates – of these, the best characterized is the transcription factor NFAT. Calcineurin is a heterodimer, which consists of a catalytic subunit, calcineurin A, and a regulatory subunit, calcineurin B. In mammals there are three different genes encoding the calcineurin A subunit, calcineurin A α, calcineurin A β and calcineurin A γ. The γ form is almost exclusively expressed in the testis, and the other isoforms are expressed more ubiquitously, with the ratios of α to β differing between tissues. Calcineurin A consists of three main functional domains: a catalytic region, an autoinhibitory domain and a calcium/calmodulin binding domain (for review see [24]). Examining the known roles of calcineurin in cells may help in understanding how DSCR1 can protect cells during oxidative stress, yet contribute to chronic disease (table 1).

DSCR1 and disease

Alzheimer's disease

Since DSCR1 is a calcineurin inhibitor, and calcineurin represents a large portion (greater than 1%) of total brain protein [25], alterations in expression of the *DSCR1 (Adapt78)* gene would be expected to have a significant effect in the brain. In fact, calcineurin activity levels have been reported to decrease in Alzheimer's disease [26]. Calcineurin inhibition has been associated with tau phosphorylation at threonine 181 and 231 – these phophorylated residues are seen in paired helical filament preparations from Alzheimer's disease brains [27]. A separate study reported that calcineurin inhibition also prevented proteolysis of tau by calpain [28].

It has been proposed that a role for *DSCR1 (Adapt78)* in the development of Alzheimer's disease is that it inhibits calcineurin from dephosphorylating the tau protein, resulting in hyperphosphorylated tau, which may then promote the formation of paired helical filaments and neurofibrillary tangles [12, 29]. This corresponds to data from other studies showing decreased calcineurin activity in Alzheimer's disease and other data showing that calcineurin inhibitors result in tau being phosphorylated on serine and threonine residues, consistent with those that occur in Alzheimer's disease [30].

DSCR1 (Adapt78) and calcineurin are expressed at similar times and in similar regions of the brain. *DSCR1*

Table 1. DSCR1 (*Adapt78*) in disease.

Condition	Possible mechanism
Alzheimer's disease	DSCR1 inhibits the dephosphorylation of tau by calcineurin, leading to hyperphosphorylated tau, which can form paired helical filaments and neurofibrillary tangles. Calcineurin inhibition also leads to decreased proteolysis of tau by calpain
Cardiac and skeletal hypertrophy	DSCR1 inhibits the dephosphorylation of NFAT-3 by calcineurin. Phosphorylated NFAT cannot move to the nucleus, bind to DNA or activate genes, including genes involved in the hypertrophic response
Brain ischemia stroke	DSCR1 is induced by oxidative stress to inhibit calcineurin activation of nitric oide synthase and subsequent NMDA-mediated neurotoxicity. DSCR1 also inhibits dephosphorylation of the protein Bad. Dephosphorylated Bad would move from the cytoplasm to the mitochondria, displacing the bax/bak complex, leading to apoptosis
Immunosuppression Autoimmune disease	DSCR1 inhibits the dephosphorylation of NFAT-1 and NFAT-4 by Calcineurin, leading to decreased T cell activation and decreased cytokine production

(Adapt78) is expressed primarily in neurons in both rat and human brain tissue [12]. This complements data from rat tissue showing that calcineurin is expressed in neurons as well [31, 32]. In these studies, some of the highest levels of calcineurin and *DSCR1 (Adapt78)* were detected in the hippocampus.

In human post-mortem brain samples, *DSCR1 (Adapt78)* RNA levels are two times greater in Alzheimer's disease than in age-matched controls. Its upregulation is even more strongly correlated to one of the hallmark Alzheimer's disease pathologies, neurofibrillary tangles. These samples exhibit a threefold increase in DSCR1 levels [12].

A second hallmark of Alzheimer's disease is plaque formation, a major component of which is the amyloid beta (Aβ) peptide. The Aβ peptide forms as a result of processing and secretion of the amyloid precursor protein (APP). Formation of Aβ has also been shown to be affected by calcineurin in a cell-free system where calcineurin induces Aβ formation. The effect of this inhibition of calcineurin was confirmed in an intact cell system in this study as well [33]. It has been shown that addition of $A\beta_{1-42}$ to cell culture can also induce *DSCR1 (Adapt78)* expression [12]. Induction of *DSCR1* (*Adapt 78*) in response to Aβ could be a mechanism by which cells attempt to inhibit calcineurin to prevent further Aβ accumulation. Aβ has been shown to interfere with early as well as late long-term potentiation (L-LTP), a major phase of memory storage. In a study using Sprague-Dawley rat slices, pretreatment of these slices with calcineurin inhibitors prevented Aβ from interfering with L-LTP [34].

Down syndrome

Alterations in levels of *DSCR1 (Adapt78)* expression have not only been detected in Alzheimer's disease patients, but are found in Down syndrome patients who also suffer from an early onset form of Alzheimer's disease [6, 12]. *DSCR1 (Adapt78)* lies adjacent to the section of chromosome 21, whose trisomy is believed to be the minimal requirement for the expression of the Down syndrome phenotype, although other regions of the genome may add to the severity of expression of the disease [14]. Besides being located in a region of the genome which is a candidate for genes implicated in Down syndrome, expression of *DSCR1 (Adapt78)* is high in brain and heart, two tissues significantly affected in Down syndrome [2, 3, 12, 15]. In fact, the expression of *DSCR1 (Adapt78)* is roughly two times greater in Down syndrome than in control brain tissue [6, 12]. Patients with Down syndrome exhibit pathologies that are thought to be the result of improper gene dosages, as well as effects downstream of these trisomys.

In model systems that may mimic the improper dosage of *DSCR1* (*Adapt78*), aberrations that resemble disease pathology are seen [15]. It is not unreasonable to hypothesize that these abnormalities are the result of the direct *DSCR1 (Adapt78)* inhibition of calcineurin, since Down syndrome is, in fact, caused by improper gene dosage. There are three major defects that are seen in Down syndrome patients: DS patients exhibit neuropathological features of early onset Alzheimer's disease (before the age of 40) (reviewed in [35]), DS patients display cardiac defects (reviewed in [36]) and DS patients present immunological dysfunctions (reviewed in [37]). Calcipressin and calcineurin are present in all of these systems, and

the balance of these two proteins could result in similar problems.

Brain ischemia/stroke

Animal models of cerebral ischemia are used to mimic the damage caused by stroke. There are two general types of models employed: global type models, which may be more closely related to cardiac arrest, and focal models, thought to be more closely related to human stroke. Stroke models may consist of permanent occlusions or may consist of a transient occlusion followed by reperfusion, the latter of which is thought to more closely resemble stroke (for review see [38]).

During prolonged ischemic injury ATP is depleted, being eventually converted into hypoxanthine, while intracellular calcium levels rise. Xanthine dehydrogenase is converted into xanthine oxidase, which produces superoxide radicals and hydrogen peroxide upon tissue reperfusion with oxygen. Since *DSCR1 (Adapt78)* is induced by both by calcium and hydrogen peroxide during adaptation to oxidative stress, it is also likely induced during this type of injury [5, 21]. Similar adaptation to ischemic injury has also been seen using ischemic preconditioning [39, 40]. It has also been noted that regions of the brain most vulnerable to ischemia have some of the highest levels of calcineurin [31, 41–43].

Calcineurin inhibitors have been observed to be neuroprotective against ischemic insults, and these inhibitors should prove to be quite helpful in elucidating calcipressin's physiological role [44–52]. There has been some controversy in the efficacy of these drugs in different models of ischemia. In several models of ischemic embolic stroke, calcineurin inhibitors have been shown to be neuroprotective against reperfusion injury [46, 50, 51, 53], but in other model systems this protection has not been seen [44, 49, 52].

Some of the controversy in the differing protection of calcineurin inhibitors in ischemia studies might result from the different amounts of drug used as well as different methods of application. FK506 readily crosses the blood-brain barrier, but this is not the case with cyclosporin A. In a study using a radiolabeled cyclosporin A, only about 5% of the drug was seen to cross the blood-brain barrier. Some studies have used 50 mg/kg of cyclosporin A, which is thought to overwhelm the blood-brain barrier, allowing some of the drug to cross. This, however, is a very toxic dose of the drug, and it is arguable that some of the differences in this model may be due to side effects of its toxicity [52, 53].

Another method that has been used to get the drug across the blood-brain barrier is to make a lesion in the barrier with a needle, and then treat with the drug at a less toxic concentration. This may be a more appropriate method of application; in fact, in work by Uchino et al. this disruption of the blood-brain barrier showed more protection against damage to the hippocampal CA1 region than did cyclosporin A administered without blood-brain barrier disruption [49]. A more recently used technique for drug administration is to inject the drug into the carotid artery, which also allows the drug to cross the blood-brain barrier [51, 52, 54]. Studies also differ in whether inhibitors are applied pre or post ischemic injury, and in the time frame of the drugs application. In a study by Yoshimoto et al., using post treatment with cyclosporin A in a transient focal ischemia rat model, an application of 10 mg/kg with permeation of the blood-brain barrier reduced the infarct volume to 40% of controls, and 50 mg/kg reduced the infarct volume to 20% of controls (but with significant toxicity). Injection of Cyclosporin A at 10 mg/kg into the carotid reduced the infarct volume to 10% of controls, but 5 mg/kg injected into the carotid did not show significant reduction in the infarct volume [52]. It has also been shown that FK506 at a dose of 2 mg/kg shows less protection than a dose of 1 mg/kg [50]. These results illustrate the importance of dosage and administration in the outcome of experiments using calcineurin inhibitors.

There may be several pathways by which the inhibition of calcineurin is protective against ischemia and/or ischemic reperfusion injury. One pathway may involve nitric oxide synthase. Nitric oxide synthase is known to be a substrate of calcineurin, and is activated upon dephosphorylation. Calcineurin inhibitors can block this activation and the subsequent production of nitric oxide. Nitric oxide synthase inhibitors can block N-methyl-D aspartate (NMDA) neurotoxicity, and low concentrations of cyclosporin A and FK506 have been shown to reduce NMDA-mediated glutamate neurotoxicity in cell culture [55]. Inhibition of nitric oxide synthase by 7-nitroindazole (7-NI) has been shown to decrease infarct volume as well, while inhibition of both neuronal nitric oxide synthase and endothelial nitric oxide synthase have been shown to increase infarct volume after 60 min of ischemia in vivo, perhaps due to vasoconstriction [56]. Some of the differences between inhibitors may be due to the fact that cyclosporin A can inhibit the mitochondrial permeability transition pore, while FK506 cannot. Blocking this pore prevents mitochondrial swelling, uncoupling of oxidative phosphorylation, rupture of the outer membrane and subsequent cell death. This action is independent of calcineurin inhibition, and studies that use both inhibitors, or the nonimmunosuppressive cyclosporin A analogue N-methyl-Val-4-cyclosporin A, may provide better insight into the effects of calcineurin inhibition.

High levels of calcineurin have been shown to predispose neuronal cells to apoptosis, and inhibition of apoptosis may be another mechanism by which calcineurin inhibitors protect against ischemia [57]. The protein bad is known to be a substrate for calcineurin [58]. Bad is phos-

phorylated and bound to the 14-3-3 protein in the cytoplasm under normal conditions. When bad is dephosphorylated by calcineurin, it can move from the cytoplasm to the mitochondria, displacing the bax/bak complex and inducing apoptosis. Blocking this effect with calcineurin inhibitors may ameliorate damage during ischemia and reperfusion injury.

Cardiac and skeletal muscle hypertrophy

Cardiac muscle hypertrophy is an adaptive (functional hypertrophy) or maladaptive (pathologic hypertrophy) response in which cells increase cardiac output, by increased cell size and strength without an increase in cell number, and a shift toward expression of fetal isoforms of genes. Calcium has been noted as a signal for hypertrophy, and calcineurin was implicated as a player in hypertrophy by Molkentin et al. who were looking for factors interacting with GATA4, a cardiac zinc finger transcription factor, during hypertrophy [59]. Using a yeast two-hybrid system, NFAT3 was identified as a GATA4 binding protein, and it was later shown that these proteins induced B-type natriuretic peptide (BNP) gene transcription, which is a marker of a hypertrophic response (fig. 2). The authors went further and showed that mice that express either constitutively active calcineurin, or NFAT3, in the heart develop cardiac hypertrophy. In these model systems calcineurin inhibitors have been observed to prevent hypertrophy in response to angiotensin II and phenylephrine, as well as the downstream occurrence of dilated cardiomyopathy [59]. Dephosphorylated NFAT also enhances the binding of myocyte enhancer factor-2 (MEF-2) to DNA, and the transcription of skeletal muscle slow fiber specific genes [60–62].

More recently, a complementary result has been shown in a line of mice with the calcineurin A β subunit disrupted. In this line of calcineurin A β knockout mice, calcineurin activity decreased 80% in the heart, and mice had a 12% reduction in heart size [63]. It is quite possible that the decreased heart size is due to a slight inhibition of calcineurin A β during normal developmental hypertrophy and is present prior to stress stimulation. Since calcineurin is known to be a key player in hypertrophy, there have been several studies examining the effects of the calcineurin inhibitors cyclosporin A and FK506 on different models of hypertrophy. Some of these models have shown prevention of hypertrophy [64–68], while others have failed to inhibit hypertrophy [69, 70]. It is likely that these differing results are due to the type of hypertrophic stimuli, as well as the dosage of the inhibitor. Calcineurin also participates in other signaling cascades, and systemic dosage of these inhibitors is likely to affect other pathways too.

A line of transgenic mice carrying a cardiac-specific promoter and a *DSCR1 (Adapt78)* transgene also have a

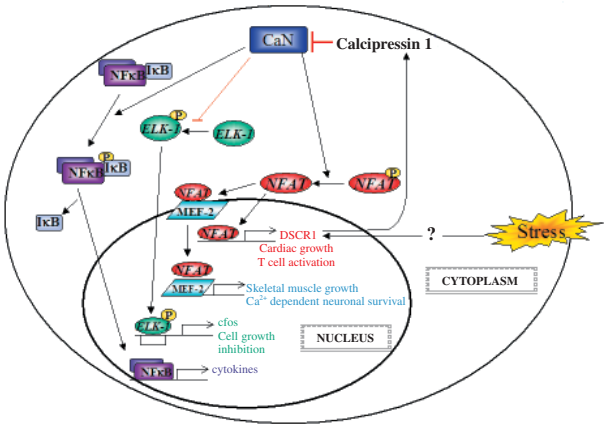

Figure 2. Calcineurin (CaN) is known to positively or negatively regulate transcription factors by removing phosphate groups, which, in turn, regulates gene expression. *DSCR1* (*Adapt78*) inhibits calcineurin and should, therefore, alter this regulation. This scheme gives a few generalized mechanisms to show how *DSCR1 (Adapt78)* may alter gene expression and may contribute to mechanisms involved in disease. NFAT: nhibition of NFAT dephosphorylation by *DSCR1* (*Adapt78*) prevents transcription of several genes. The genes controlled by NFAT depends on the form of NFAT that is present. For example, NFAT3 is responsible for transcription of genes involved in cardiac hypertrophy, and NFAT1 and NFAT4 are responsible for transcription of genes involved in the immune response. *DSCR1* (*Adapt78*) also inhibits the transcription of itself through the calcineurin/NFAT pathway, functioning in a negative feedback loop. ***MEF-2*** (myocyte enhancer factor-2): *DSCR1* (*Adapt78*) should also inhibit skeletal muscle growth and calcium-dependent neuronal survival through calcineurin via the NFAT-stimulated binding of MEF-2 to DNA. ***Elk-1:*** *DSCR1* (*Adapt78*) should release the inhibition of Elk-1, allowing it to move to the nucleus where it can bind to DNA and transcribe *c-fos* and genes involved in cell growth inhibition (early response genes). ***NFκB:*** Calcineurin stimulates NFκB (p50/cRel) translocation into the nucleus by inactivation of the inhibitory protein IκB. *DSCR1* (*Adapt78*) should inhibit this effect, as well as the inflammatory and immune response stimulated by NFκB binding to DNA.

5–10% decrease in heart mass relative to their total mass and have defects in heart valve formation, but no other apparent abnormalities [71]. As would be expected, these mice have an inhibited hypertrophic response induced by β adrenergic stimulation as well as by exercise training. In mice carrying inducible, cardiac-specific versions both of this transgene and a constitutively active calcineurin transgene, there is only 28% of the response as seen with animals carrying the active calcineurin construct alone, and dilated cardiomyopathy is prevented.

DSCR1 (Adapt78) has been implicated in cardiac valve formation and cardiac hypertrophy, as well as skeletal muscle hypertrophy [10, 13]. Calcineurin has been shown to be involved in skeletal myoblast hypertrophy, downstream of insulin-like growth factor-1 (IGF1). This hypertrophy can be blocked by application of cyclosporin A [72]. Active calcineurin leads to hypertrophic growth, which is attenuated by the endogenous

calcineurin inhibitors Cain and A-kinase anchoring protein (AKAP) [73]. By directly interacting with and inhibiting calcineurin, *DSCR1 (Adapt78)* can prevent NFAT from translocating to the nucleus and binding to the GATA4 and MEF-2 promoters [11], thus preventing hypertrophy.

Immune system disorders and immunosuppression

Although the effects of *DSCR1 (Adapt78)* on the immune system have not yet been directly examined, the therapeutic benefits of other calcineurin inhibitors have been examined for a variety of conditions. The two most commonly used clinical calcineurin inhibitors are cyclosporin A and FK506, which are widely used as anti-rejection drugs for organ transplant patients. Their main mechanism of action is believed to be immunosuppression via inhibition of calcineurin. By preventing the dephosphorylation NFAT in the cytoplasm, these drugs block the translocation of NFAT to the nucleus and, thereby, inhibit transcription of genes involved in T cell activation. In the case of T cells many of the genes that would be induced by NFAT are cytokines involved in the immune response (reviewed in [74]). Calcipressin should provide protection against the effects of autoimmune diseases involving the T cell response, or events occurring downstream of this response.

The NFκB protein functions as a transcription factor that can elicit an inflammatory immune response. NFκB remains in the cytoplasm when bound to the inhibitory IκB protein, translocating to the nucleus and stimulating transcription only when IκB is removed. Calcineurin can dephosphorylate IκB, leading to the subsequent translocation of NFκB (p50/cRel) into the nucleus. *DSCR1 (Adapt78)* should, therefore, inhibit the inflammatory and immune response stimulated by NFκB-dependent transcription [75–77].

Since intracellular calcineurin concentration varies with cell type, the dosage of inhibitor required for a therapeutic effect may be excessive in some tissues, or for chronic conditions. The side effects of these drugs, notably nephrotoxicity and neurotoxicity, are a major drawback, and the possibilities of using *DSCR1 (Adapt78)* as an endogenous therapy are exciting, especially if its expression can be tightly regulated. Dosages of the currently used drugs may have critical levels, because although they do show promise for treatment of some autoimmune disease, there are often conflicting results for the use of the drugs in the very same diseases.

Not only could *DSCR1 (Adapt78)* be beneficial to organ transplant patients, but it could also provide therapies for other autoimmune diseases that have shown some successful results when treated with calcineurin inhibitors. Cyclosporin A is currently approved for use in rheumatoid arthritis, psoriasis, atopic dermatitis, uveitis, aplastic anemia, nephrotic syndrome and has some promise/possibilities for treatment of other autoimmune diseases (see table in [78]). With the current inhibitors of calcineurin, the costs outweigh the benefits of the therapy for many conditions.

A study compiling data from clinical experience using cyclosporin A as a treatment for a variety of autoimmune disorders reports the success of this drug in different diseases. The success of the drug varied with the disease being treated. The greatest success was achieved in the treatment of Behcet's disease, with 16 of 18 patients obtaining a full therapeutic response (remission). Less success was achieved in treating systemic lupus erythematosis and vasculitis of the central nervous system [79].

A role for calcineurin has been proposed in rheumatoid arthritis as well, and cyclosporin A has been approved for clinical use in this disease. In a double blind study, patients who were not responding well to treatment with methotrexate – either because of resistance to the drug, or intolerance of it – were treated with FK506. There were initially 268 patients who started the study and there were 141 patients who completed it. The treatments lasted for 24 weeks, and patients were treated with placebo, 1mg, 3mg or 5-mg doses of FK506 once daily. The ACR20 (American College of Rhematology definition of 20% improvement) and tender and swollen joint counts at the resolution of the study gauged success of treatment.

Cyclosporin A has also been shown to effect cytokine production in cultured fibroblast like synoviocytes taken from patients suffering from rheumatoid arthritis. When these cells were treated with cyclosporin A, interleukin (IL)-10 production increased and IL-15 and tumor necrosis factor α (TNFα) production decreased, both at the mRNA and protein levels. Decreases in IL-15 and TNFα in response to cyclosporin A inhibition were also seen with stimulation via interferon γ, IL-1β or lipopolysaccharide. These increases were dependent on IL-10 levels and camp, and FK-506 gave similar results [80].

Diabetes mellitus

Calcineurin has also been implicated in diabetes. Diabetes affects the kidneys, and the pathology of diabetes in this organ includes hypertrophy and extracellular matrix expansion, which has been shown to depend on calcineurin in a cell culture model [81]. In recently published work, the role of calcineurin was examined in a rat model of type I diabetes. Rats were injected with streptozotocin to induce diabetes, and were also injected with or without the calcineurin inhibitor cyclosporin A (along with appropriate controls). They found that levels of both calcineurin protein and activity increased in the diabetic model, but decreased when treated with a calcineurin inhibitor. More important was the finding that cyclosporin A also inhibited kidney hypertrophy [82]. The NFAT pathway

has been shown to be a calcium-responsive mechanism that regulates glucagon synthesis in pancreatic islet cells, and in turn regulates blood glucose levels [83]. Another hint that *DSCR1 (Adapt78)* could play a role in diabetes comes from the finding that *DSCR1 (Adapt78)* is induced in response to 2-deoxyglucose [17].

Dermatological Diseases

Pharmacological calcineurin inhibitors have shown success in the treatment of a number of dermatological conditions (for review see [84]). Although both FK506 and cyclosporin A can be delivered orally, FK506 is a small molecule and can be administered topically and therefore may be preferred over cyclosporin A for dermatological use in some instances, unless lesions are present. Cyclosporin A typically has a greater risk-to-benefit ratio, and this is another reason FK506 may be preferred.

The dermatological condition most commonly treated with pharmacological calcineurin inhibitors is psoriasis. Both FK506 and cyclosporin A have been shown to be clinically effective in repairing lesions, reducing hyperkeratosis and decreasing inflammation in affected areas [85, 86]. Psoriasis is characterized by keratinocyte proliferation and hyperplasia, by abnormal differentiation and by the presence of activated neutrophils and lymphocytes in the dermis and epidermis. Both calcineurin and NFAT have been found to be expressed in a variety of skin cell types in both normal and psoriatic skin samples, particularly in epidermal keratinocytes, with greater activation of calcineurin in psoriatic epidermal keratinocytes [87].

Inhibition of calcineurin by both FK506 and cyclosporin A has improved a variety of other skin ailments as well. In a compilation of patients treated for scleroderma with various regimens of FK506 or cyclosporin A, patients showed improvement, although a large percentage of patients had side effects [88].

Conclusions

DSCR1 (Adapt78) may be involved in the regulation of several different disease pathways, including neurodegenerative diseases such as Alzheimers's disease and Down syndrome, cardiac hypertrophy, and autoimmune diseases such as rheumatoid arthritis and psoriasis. Learning how *DSCR1 (Adapt78*) is expressed and regulated in a variety of tissues and under different conditions may shed light on these conditions, and might even lead to effective therapies. The prospect of an endogenous calcineurin inhibitor that could be used as a genetic antirejection therapy, perhaps without the serious side effects of the current therapies, is very exciting.

Although the *DSCR1 (Adapt78*) protein product is a calcineurin inhibitor, it may also have other functions, and its effects may differ from those of the pharmaceutical calcineurin inhibitors. Indeed, as shown by Cunningham et al. [23], the protein may inhibit calcineurin at some concentrations and stimulate calcineurin signaling at other concentrations. Based on these results, it may actually be advisable to name the gene *RCAN1* (for Regulator of Calcineurin 1) and to call the protein rcan1. Whatever name we use, this gene is clearly inducible by multiple stresses, and provides a link between stress and disease. The possibilities for *DSCR1 (Adapt78*)'s involvement in the conditions presented here are primarily based on what is known about calcineurin pathways and pharmaceutical calcineurin inhibitors. Much more research is needed to fully understand how *DSCR1 (Adapt78*) functions in these diseases, but the potential impact is very interesting.

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