

# Renaming the *DSCR1/Adapt78* gene family as *RCAN*: regulators of calcineurin

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to modulate calcineurin activity under physiological and pathological conditions. Unfortunately, the members of this family have been given some 20 different

## INTRODUCTION AND SIGNIFICANCE

THE SERINE-THREONINE PHOSPHATASE, calcineurin (also known as PP3C, formerly PP2B) plays pivotal roles in a wide series of key biological processes. A new family of regulators of calcineurin (RCANs) has been shown

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names over the past 10 years largely because their function as calcineurin regulators was discovered only recently. These diverse names have resulted in great confusion, and still represent a barrier to advancement in the field. In writing this paper, our goals were 1) to bring together all the previous names for members of this RCAN family in one publication, where their identities can be easily compared; 2) to introduce RCAN as a rational, functional name for this gene family; and 3) to propose a coherent nomenclature system for the known RCAN family members, as well as additional ones that may be found in the future.

## PROPOSAL

In the interest of advancing work in the field, we propose a unified nomenclature for the family of genes related to the locus initially designated as human *DSCR1*. The new *RCAN* nomenclature has been approved by both the HUGO Gene Nomenclature Committee (HGNC) and the Mouse Genomic Nomenclature Committee (MGNC). Homologs, orthologs, and paralogs of this gene, and their protein products, should now be named “regulators of calcineurin” to reflect the ability of most family members to bind and regulate the protein phosphatase enzyme, calcineurin (see **Table 1**). The gene names would be abbreviated as *RCAN* (*Rcan* for mouse and rat) and their protein

products as *RCAN* (*Rcan* for rat and mouse). The original *DSCR1* locus on human chromosome 21 would be designated *RCAN1*. Because all yeast and fungal genes and proteins are named with only three letters and usually a number, regulators of the calcineurin genes would be referred to as *RCNI*, and the proteins as *RCN1*, in these species.

The *RCAN1* gene consists of seven exons, of which exons 1–4 can be alternatively transcribed or spliced to produce different mRNA isoforms. In the new nomenclature, two identified *RCAN1* protein isoforms would be designated *RCAN1–1* and *RCAN1–4* (see **Fig. 1**). Similarly, the homologous gene locus on human chromosome 6 would be designated *RCAN2* (protein isoforms *RCAN2–3* and *RCAN2–4*). The family member on human chromosome 1 would be designated *RCAN3*. Orthologs from other mammalian species would follow the same numbering scheme based on their relative identity to these three human family members. Structural homologues from species containing only one *RCAN* family member would be named simply *RCANI*, with additional numbered genes added if new loci are identified. When conversing, *RCAN* may be easily referred to as “R-can” (pronounced “ar-can”). This new naming system provides information regarding an important function of the *RCAN* genes and proteins, and will make it much easier for outside researchers to access the literature and grasp the potential importance of these highly conserved regulators of calcineurin signaling.

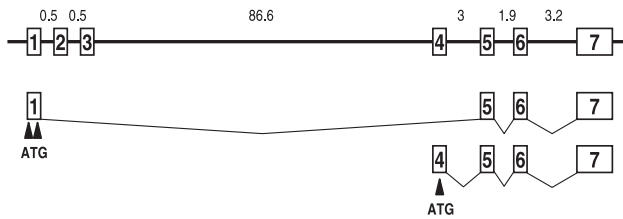
TABLE 1. A historical overview of the existing *RCAN* gene nomenclature

Name	Source	Reference	Year
The <i>RCAN1</i> gene			
<i>DSCR1</i>	<i>Homo sapiens</i>	(1)	1995
	<i>Saccharomyces cerevisiae</i>	(2)	2000
	<i>Caenorhabditis elegans</i>	(2)	2000
<i>Dscr1</i>	<i>Mus musculus</i>	(3)	2000
<i>Adapt78</i>	<i>Cricetulus griseus</i>	(4)	1997
	<i>Homo sapiens</i>	(5)	2001
MCIP1	<i>Mus musculus</i>	(6)	2000
	<i>Homo sapiens</i>	(6)	2000
Calcipressin1 [Csp1,CALP1]	<i>Homo sapiens</i>	(7) [(8, 9)]	2000 [2003, 2003]
<i>RCN1</i> , <i>Rcn1p</i>	<i>Saccharomyces cerevisiae</i>	(10)	2000
CBP1	<i>Cryptococcus neoformans</i>	(11)	2000
RCN-1	<i>Caenorhabditis elegans</i>	(12)	2003
<i>Nebula (nla)</i>	<i>Drosophila melanogaster</i>	(13)	2003
Sarah ( <i>sra</i> )	<i>Drosophila melanogaster</i>	(14)	2004
RCAN1	<i>Homo sapiens</i>	(15)	2005
The <i>RCAN2</i> gene			
ZAK1-4	<i>Homo sapiens</i>	(16)	1996
<i>Dscr111</i>	<i>Mus musculus</i>	(2, 3)	2000
MCIP2	<i>Mus musculus</i>	(17)	2000
Calcipressin 2	<i>Mus musculus</i>	(18)	2006
The <i>RCAN3</i> gene			
DSCR1L2	<i>Homo sapiens</i>	(2)	2000
<i>Dscr112</i>	<i>Mus musculus</i>	(3)	2000
RCAN3	<i>Homo sapiens</i>	(19)	2007

Table 1 is an attempt to bring together all the published names for the *RCAN* family of genes to demonstrate the relationships between family members. Only the first published use of a name (to the best of our knowledge) is referenced in each species or genus. The [Csp1,CALP1] variations on calcipressin 1 (and their references) appear in brackets to indicate they were modifications of an existing proposed name.

## Gene name and reference

### *RCAN1* (References 9,17,23,24,26,31)



### Protein Name

### Location

RCAN1-1

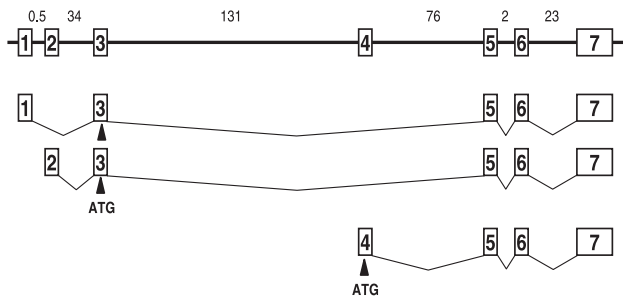
Human: Chr 21

Mouse: Chr 16

RCAN1-4

Rat: Chr 11

### *RCAN2* (Reference 34)



RCAN2-3

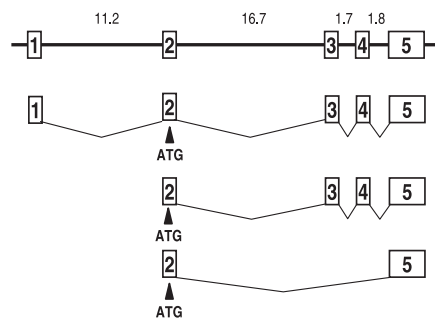
Human: Chr 6

Mouse: Chr 17

Rat: Chr 9

RCAN2-4

### *RCAN3* (References 2,35)



RCAN3-2

Human: Chr 1

Mouse: Chr 4

Rat: Chr 5

RCAN3-2,3,4b,5

RCAN3-2,5

**Figure 1.** The intron/exon structure, transcripts, and protein products of the human *RCAN* genes. Overall: Note that all structures, exon locations, and protein isoform nomenclature are based on that of the human *RCAN1* gene. Genomic distances between exons (*i.e.*, intron sizes) are given in kilobases. *RCAN1*: Protein isoforms have been named based on the (human) exon location of the translation start site. In the new nomenclature, the various *RCAN1* transcripts corresponding to alternative first exons would be designated *RCAN1-1*, *RCAN1-2*, *etc.*, as previously proposed (31). Exons 5, 6, 7 are omitted from the mRNA transcript and protein isoform nomenclature above. This is possible since exons 5, 6, 7 are invariant components of all transcripts so far observed. The transcript encoding *RCAN1-1* starts at exon 1 and its expression is stimulated by glucocorticoids. The transcript encoding *RCAN1-4* begins at exon 4 and its expression is controlled by oxidative stress, calcium, and calcineurin. One long (*RCAN1-1L*) and one short (*RCAN1-1S*) version of the *RCAN1-1* protein are generated due to alternative translation initiation sites in the *RCAN1-1* transcript. *RCAN2*: the system of nomenclature used for each mRNA transcript and protein isoform is the same as that proposed for *RCAN1*. The *RCAN2-1,3* and *RCAN2-2,3* transcripts both produce the same protein: *RCAN2-3*. This is because the first initiation codon lies within exon 3. It should be noted that *RCAN2-4* (but not *RCAN2-1,3* or *RCAN2-2,3*) is up-regulated by the thyroid hormone. *RCAN3*: the system of nomenclature used for each mRNA transcript and protein isoform is similar to that proposed for *RCAN1*. Exon 4b (an exon 4 variant lacking the first 30 bases, leading to a product lacking 10 amino acids) makes it necessary to specify the particular exon included in the *RCAN3-4b* mRNA isoform. Exon 1 has been redefined as described by Canaider *et al.* (35); the first initiation codon lies within exon 2. The originally published name for *RCAN3-2,5* was *DSCRIL2-E2E5* (35), and it has been reported that proteins encoded by these mRNA transcripts interact with cardiac troponin I (*TNNI3*) (35) and calcineurin (19).

## THE *RCAN* GENE FAMILY

*DSCR1/RCAN1* was first identified by the group of Estivill as an expressed sequence on a YAC clone from human chromosome 21 (1). The YAC clone contained a portion of the chromosome known as the Down

syndrome critical region (*DSCR*) (20, 21). Thus, the name "Down syndrome candidate region 1" gene (originally designated "critical region 1" gene), abbreviated as *DSCR1*, was used for the first previously unidentified gene on this YAC clone. Although we are suggesting a new function-based nomenclature, the relationship of

*RCAN1/DSCR1* to human Down syndrome still appears strong. Other open reading frames identified from this region became designated as *DSCR 2/3/4* (or A/B/C), *etc.*, in the literature until functional data could be linked with their respective activities. Two major protein isoforms of 252 (*RCAN1-1*) and 197 (*RCAN1-4*) amino acids are expressed from the *RCAN1* gene by alternative promoter usage and first exon choice in humans and the mouse (9, 22–24). The proximal promoter controlling expression of *RCAN1-4* is activated by calcineurin-NFAT signaling (17) in response to different stimuli such as VEGF (25, 26) and depolarization in neurons (27), among others. The distal promoter (*RCAN1-1*) is activated by glucocorticoids (28) and down-regulated by Notch-dependent signaling (29).

Important insights into the regulation and function of *RCAN1/DSCR1/Adapt78* came when the Davies laboratory demonstrated that its expression was transiently induced during cellular adaptation to oxidative stress and calcium stress (4). They named the gene *Adapt78* and went on to show that it is an oxidant stress response gene and a calcium stress response gene that can protect cells from various forms of stress (30, 31). A critical breakthrough came when it was demonstrated that the protein could bind to and inhibit the calcium-regulated protein phosphatase calcineurin in yeast (10), mouse (6), human (7), and *Cryptococcus neoformans* (11). The yeast homologue was named *RCN1* for “regulator of calcineurin 1” (10). The mouse ortholog was officially designated *Dscr1* (22), but its protein product was published as MCIP1 (6) for “myocyte-enriched calcineurin-interacting protein 1” and later “modulatory calcineurin interacting protein” (32). The gene from *C. neoformans* was named *CBP1* for calcineurin binding protein 1 (11). The *Drosophila* homologue has been published with a variety of names, including *NEBULA* (*nla*) for the gene (13) and *sarah* (*sra*) for the protein (14). The *Caenorhabditis elegans* gene has been named *RCN-1* (12).

The human *RCAN2* locus was first published as *ZAKI-4* (16), a gene whose expression was induced in response to thyroid hormone. *ZAKI-4* was later designated *DSCRIL1* for “*DSCR1*-like protein 1” (2). As shown in Fig. 1, three transcripts (*RCAN2-1,3*, *RCAN2-2,3*, and *RCAN2-4*) and two protein products (*RCAN2-3* and *RCAN2-4*) have been identified (33). It has also been noted that the *RCAN2-4* protein isoform is up-regulated by thyroid hormone (33) *via* an AKT/PKB-dependent signaling pathway (34). The *RCAN3* gene was identified on the basis of its sequence similarity to *RCAN1* and *RCAN2*, and was named *DSCRIL2* (2). Alternatively spliced *RCAN3* isoforms have also been described (2, 35). *MCIP 1, 2*, and *3* standing for “modulatory calcineurin-interacting proteins” were also suggested as a naming scheme for the three mammalian genes (32, 36).

This multiplicity of gene and protein names (see Table 1) is awkward and highly confusing to those outside the field. The earlier names also provide no functional information and are difficult to apply to

genes from diverse species. Based on its ability to inhibit calcineurin, the term “calcipressin” has been suggested (7, 11), and has been abbreviated in a variety of ways, including *Csp* (8) and *CALP* (9). More recently, however, it was demonstrated that *RCAN1* protein appears to be capable of either inhibiting or facilitating calcineurin signaling depending on the context (36–38). Thus, the name “calcipressin 1” may not accurately reflect the full biological functions of these proteins. Furthermore, the similarity of the name calcipressin to the calcium binding proteins calretinin, calbindin, and calsequestrin implies calcium binding properties, yet there is no evidence that *RCANs* bind calcium. Therefore, the former designations all seem inadequate when compared with *RCAN*.

## REGULATION OF CALCINEURIN BY *RCANs*

Several laboratories have reported that animal and fungal *RCAN1* and *RCAN2* can specifically bind to and down-regulate the activity of calcineurin (6, 7, 10, 11, 33). Calcineurin is a protein phosphatase enzyme composed of calcineurin A (CnA) and calcineurin B (CnB) subunits. The CnA subunit has a catalytic domain, a CnB binding domain, and a C-terminal regulatory domain. In the absence of calcium, the protein phosphatase calcineurin is inactive because of a C-terminal autoinhibitory domain (“AID”) that blocks the active site in the catalytic CnA subunit. The binding of a calcium/calmodulin complex causes a conformational change that removes the AID, thus “activating” the protein phosphatase activity.

It has been shown that *RCAN1* and *RCAN2* bind calcineurin at or near the catalytic domain of CnA (6, 7, 10). The primary calcineurin binding portion of the *RCAN1* protein is encoded by exon 7 (7, 39), which is common to all *RCAN1* isoforms. This region includes two *RCAN1* sites of interaction with calcineurin. One is a PxIxxT motif that resembles a PxIxxIT calcineurin-interacting sequence found in NFAT proteins (40). The second is an ELHA motif that binds to calcineurin but may need to be in a wider amino acid context, such as the CIC motif, in order to inhibit calcineurin (41). Two additional sites for calcineurin interaction have been reported. One is specific to the N-terminal region of *RCAN1-4* (42). The other is the highly conserved serine-proline (SP) motif (known also as a FLISPP motif), which is a calcineurin substrate. It has been demonstrated, however, that the SP motif is neither sufficient nor required for the inhibition of calcineurin, although it can act as a competitive inhibitor (9, 38, 39, 41, 42). The state of phosphorylation of the *RCAN1* SP motif correlates with the protein half-life (9). *RCAN* binding to calcineurin does not interfere with binding of either the calcium/calmodulin complex or the regulatory B subunit to the catalytic A subunit of calcineurin. In fact, most data indicate that *RCAN1* and *RCAN2* bind preferentially to the activated form of calcineurin, suggesting that calcium/calmodu-

lin binding facilitates RCAN binding by removing the AID to expose the catalytic domain.

There are ample data indicating that RCAN1 and RCAN2 can bind calcineurin and that increased RCAN levels can inhibit calcineurin's protein phosphatase activity. Nevertheless, some of the phenotypes observed in yeast, *C. neoformans* (43), and mouse mutants lacking RCAN1 or RCAN2 are similar to those observed in mutants lacking calcineurin (18, 36). This suggests that RCANs may also be required for proper calcineurin signaling. There is genetic evidence from yeast indicating that phosphorylation of the conserved serine 108 of RCAN-1–4 by GSK-3 may release the inhibitory activity of RCAN and somehow facilitate calcineurin signaling (37). Similar results have been reported in fungi (43) and mammals (44). Although the exact mechanism of this process is not known, it has been suggested that GSK-3-phosphorylated RCAN may act on calcineurin in a manner similar to the chaperoning properties of phosphorylated inhibitor-2, relative to the protein phosphatase PP1 (37). In the case of mammalian RCAN1, the switch seems to depend on 14–3–3 binding (44). RCAN3 (formerly DSCRIL2) has been shown to bind cardiac troponin I (TNNI3) (35), and very recently evidence was presented that it also binds calcineurin and inhibits NFAT-dependent gene expression (19).

#### RATIONALE FOR ACCEPTANCE OF A COMMON NAME

Laboratories around the globe are now studying RCAN genes and their protein products. RCAN1 has been shown to inhibit cardiac hypertrophy (45), to attenuate angiogenesis and cancer (26), and to be associated with Down syndrome and Alzheimer disease (5, 7, 24, 46–50). RCAN1-dependent inhibition of calcineurin and induction of GSK-3 $\beta$  expression may play important roles in the tau hyperphosphorylation seen in tauopathies such as Alzheimer disease (5, 24, 46, 48). Key roles for RCAN1 in human physiology and pathology are rapidly emerging (15). As with many newly discovered genes and proteins, initial confusion over nomenclature often hinders or obscures progress. In this case, the term *DSCR1* for Down syndrome candidate or critical region 1 is misleading, because the gene is not technically inside the DSCR as initially defined. Furthermore, since calcineurin activity is fundamental to so many biological processes, it is likely that RCAN1 and other members of this family of calcineurin regulators will have important functions well beyond those related directly to Down syndrome. Fully aware that these proteins may have additional properties, we propose that the *DSCR1* gene be renamed to reflect its influence on calcineurin signaling.

We have suggested adopting “regulators of calcineurin” for this family of proteins based on the original name given in yeast because it is straightforward and recognizes the concept that calcineurin activity can either increase or decrease depending primarily

on the level of RCAN1 induction. The abbreviations *RCAN1*, *RCAN2*, and *RCAN3* will be used rather than *Rcn1* to avoid confusion with the unrelated *rcn1* gene from Arabidopsis (51) and favoring any existing name. To implement this change, we recommend that publications initially include the *RCAN* and *DSCR* designations as well as the name previously used by the investigator. We hope that all researchers in this exciting and important field will join with us in embracing this new nomenclature. FJ

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