THE PANCREAPEDIA
Exocrine Pancreas Knowledge Base
www.pancreapedia.org

MOLECULE PAGE

RCAN1

Greg Gurda

Department of Pathology, Johns Hopkins University Hospital, Baltimore, MD, USA

e-mail: ggurda1@jhmi.edu

Version 1.0, August 27, 2012 [DOI 10.3998/panc.2012.14]

Gene Symbol: RCAN1

Other names: DSCR1, MCIP1 and Calcipressin-1

1.General Background & Pathophysiological Functions of RCAN1

There are three known RCAN (Regulator of Calcineurin) genes, RCAN1, RCAN2 and RCAN3 (33). The three genes are highly conserved throughout the vertebrate lineage with an orthologous RCN gene in both *C. elegans* and *S. cervisea* (18). All three RCANs have a similar genomic exon-intron structure and are expressed as at least 2 alternative mRNA transcripts and resultant Rcan proteins (16). When overexpressed, Rcan proteins inhibit Calcineurin (CN) with an *in vitro* IC_{50} in the nanomolar range, similar to that observed for the pharmacological agents FK506 (Tacrolimus) and Cyclosporin A (CsA), but Rcan act independent of immunophilin binding (5). The best studied member of the RCAN family is Rcan1. Rcan1 is known by several names, which is a byproduct of historical circumstance, independent identification of individual genes by several different laboratories and changing perspective of their function. Alternative nomenclature of RCAN1 include: Down Syndrome Critical Region 1 (DSCR1), Adaptor protein of 78kDa (Adap78), Myocyte-enriched or Modulatory Calcineurin Interacting Protein (MCIP1), Calcipressin 1 as well as infrequently used Csp1 and CALP1.

Physiologically, the putative role of Rcan1 is to protect cells against numerous stressors involving increased $[Ca^{2+}]_i$ and formation of reactive oxygen species (30). These transient stress processes are an integral part of neurodegenerative disease, destruction of insulin-secreting β cells, maladaptive muscle hypertrophy and premature aging, among others (21).

CN represents a large portion (>1%) of total brain protein (20) and hence Rcan1 function has been most widely studied in the nervous system. Rcan1-mediated inhibition of CN activity has been associated with increased phosphorylation and reduced proteolysis of tau proteins, which form neurofibrillary tangles in Alzheimer's disease (9). Inhibition of CN may also contribute to formation of amyloid beta peptide, a hallmark of this disease (9). The human RCAN1 gene lies within the section of chromosome 21 called the Down Syndrome Critical Region (DSRC); trisomy of DSCR is a hallmark of Down Syndrome (11).

Some characteristic traits of Down Syndrome, including mental retardation, anxiety and neuromuscular coordination deficits, have been recapitulated in various mouse models of Down Syndrome as well as Rcan1 transgenic mice (2), but the exact contributions of Rcan1 to this complex phenotype remain unclear (7). Rcan1 is also induced by oxidative stress and in response to nitric oxide in animal models of ischemia/stroke. In this setting Rcan1 has been shown to attenuate NMDA-mediated neurotoxicity (8) as well as inhibit dephosphorylation of Bad and thereby reduce neuronal apoptosis (39).

Outside of the nervous system, Rcan1 plays a critical role in cardiac and skeletal hypertrophy. Rcan1 overexpression blocks pathological hypertrophy and heart failure associated with increased load induced by hypertension or aortic stenosis (32). Rcan1 also blocks cardiac hypertrophy in genetically modified animal models of constitutively active CN as well as constitutively nuclear NFATc3. Transgenic mice which express a HA-tagged Rcan1 under the control of α-MHC promoter (32) show attenuation of cardiac hypertrophy induced by constitutively active CN, β-adrenergic agents and exercise training. Overexpression of Rcan1 in the same setting of active CN also suppressed left ventricular remodeling and dysfunction following myocardial infarction (36). Rcan1 has likewise been implicated in skeletal muscle hypertrophy, in particular the response to insulin-like growth factor-1 (27). In the immune system, Rcan1 expression has been shown to regulate not only NFATs but also NFkB, whereas genetic manipulation of Rcan1 leads to immunosuppression and skewed T-cell subtype specific responses (16). Lastly, Rcan1 has now been clearly been linked to regulation of angiogenesis (3, 25, 34). Rcan1 blocks NFAT-driven expression of vascular endothelial growth factor (VEGF) and thrombin (31). Overexpression of Rcan1 in vivo reduces vascular density and growth of melanoma allografts in mice (24) as well as neovascularization of muscle infarcts. Whether the effect of Rcan1 on CN-NFAT signaling in angiogenesis occurs solely via prime drivers of angiogenesis such as VEGF and thrombin or a combination of primary and secondary effects (such as expression of matrix remodeling proteins and cell cycle components) remains to be seen.

2. Gene/protein structure and intracellular function

The RCAN1 gene is located on orthologous chromosomes 16, 11 and 21 in mouse, rat and human, respectively. It is composed of seven exons, with exons 1-3 (E1-3) proximal to the first transcriptional start site (TSS) and separated by approximately 0.5kb, followed by a long intron (~35kb), a second TSS and remaining exons 4-7 (E4-7) (11). The genomic organization of Rcan1 lends itself to formation of several alternative transcripts. Although four have been identified thus far (10), the two best described and ubiquitously expressed variants are Rcan1.1 (also known as DSCR-1L/Rcan1L), which is comprised of E1 together with E5-E7, versus Rcan1.4 (also known as DSCR-1S/Rcan1S), which is composed of E4-7 (16). Additional transcripts have been described but appear to have a very restricted pattern of expression that may be limited to embryogenesis. As examined by western blotting, Rcan1.1 and Rcan1.4 code for proteins of 48kDa and 24kDa, respectively (38). Despite their differences in size, the two are almost equally potent inhibitors of CN (29). Rcan1.4, however, contains tandem NFAT binding sites in its promoter and as shown both in our work (14) and multiple other studies (38, 40), its product is the only one among Rcan1 variants whose expression is regulated by CN-NFAT signaling. The alternative Rcan1.1 variant also inhibits calcineurin but is regulated by Notch/Hes1 and oxidative stress (25, 33).

All Rcan proteins consist of several conserved domains (Figure 1) (33). The N-terminal contains a CN-binding domain, a putative dimerization

domain that contains an amphipathic leucine repeat, as well as a central SP repeat domain analogous to those seen in NFATc1-c4. The C-terminus contains a second, more highly conserved CN-binding and inhibitory domain (33) as well as a region important for nuclear localization, which may occur either independently or in conjunction with CN. Work to better define these domains as well as the detailed experimental analysis of the functional differences between various RCAN genes and transcript variants is still underway.

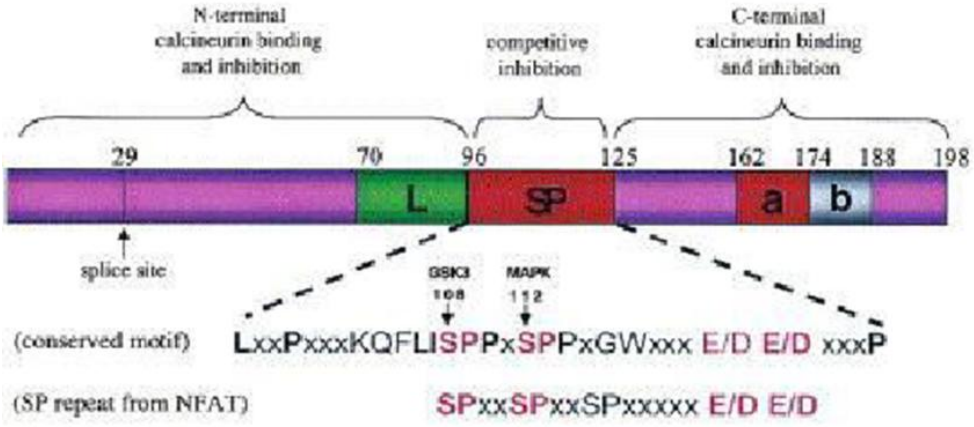

Figure 1. Structure of Rcan1 — 3 domains include N-terminal and C-terminal CN-binding domains and a conserved serine-proline (SP) repeat domain with multiple protein-protein interaction sites, including those for NFAT, GSK, MAPK and 14-3-3.

At basal $[Ca^{2+}]_i$, Rcan1 is synthesized at a low rate and stochastically binds to CN; in its free form it is rapidly degraded, possibly by the protease calpain (13). With an appropriate stimulus, such as an increase in $[Ca^{2+}]_i$, Rcan1 expression is transcriptionally upregulated due to NFAT-mediated expression of Rcan1. The excess Rcan1 is then able to bind to and inhibit CN (Figure 2) (37). Recent studies have shown that Rcan1 can also be regulated via phosphorylation at its Serine-Proline Repeat Domain (specifically Ser108 and Ser112) which modulate its CN-binding activity, subcellular localization and perhaps also its half-life (12, 23). Several kinases have been recognized to act on Rcan1, including JNK and GSK3β (19). Though still poorly understood, regulation of Rcan1 expression and function clearly link CN-NFAT signaling to parallel signaling pathways including PPAR-γ, MAPK, ATF6 and wnt/CEBP/GSK3β (26). Rcan1 and quite possibly other members of the RCAN family may also anchor/localize CN to specific intracellular domains and chaperone or facilitate its interaction with other proteins. Rcan1, for instance, can interact with the well-known scaffolding protein 14-3-3 (1) and knock-down of Rcan1 protein below basal levels has in some context interestingly shown to actually *inhibit* CN (35, 38); this suggests that some low level of Rcan1 may be necessary for CN activity. Several

important questions that still need to be addressed are the precise mechanism of Rcan1 action, the tissue or target-specific attributes of its inhibitory effect on CN, additional signals that regulate its expression and function as well as additional intracellular functions independent of its effect on CN.

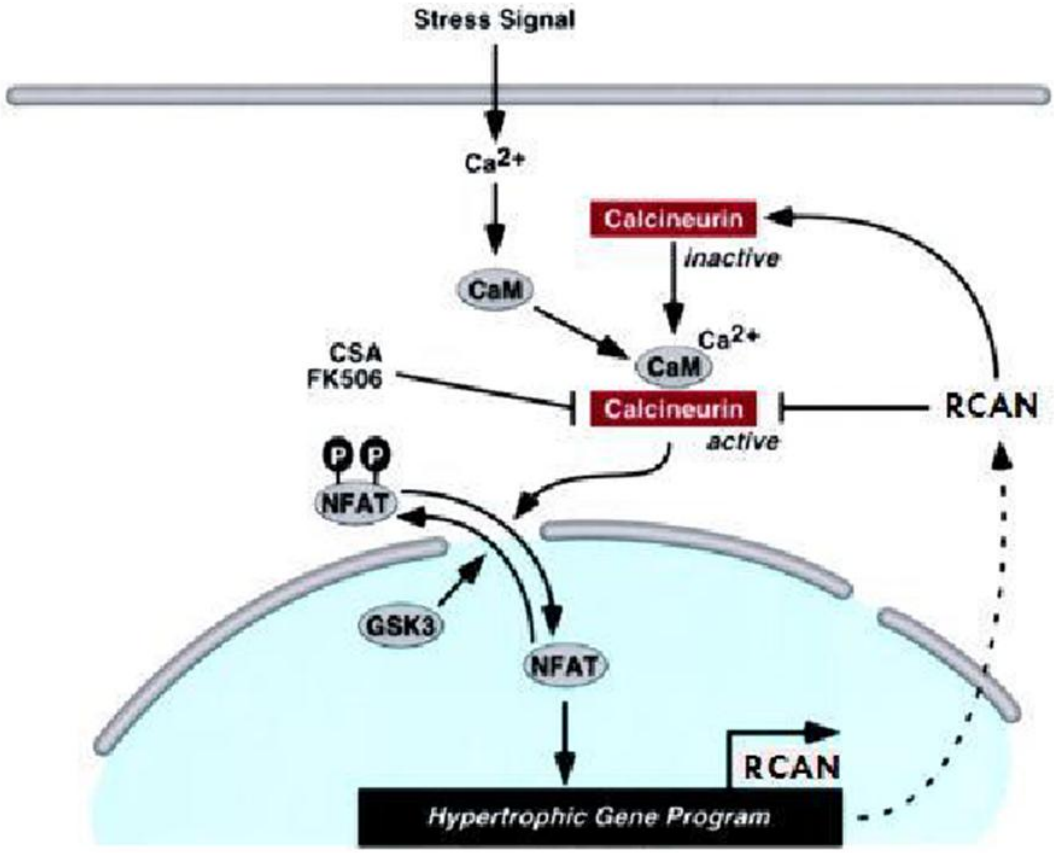

Figure 2. Overview of the CN-NFAT-Rcan1 pathway (adapted from Vega et al, reference 38).

3.Rcan1 function in the pancreas

Based on searches of the pubmed database and disease & tissue atlas (http://www.nextbio.com), our group was the first to carefully explore Rcan1 specifically in the exocrine pancreas. In our past work, we had shown that Rcan1 is the only member of the RCAN family to be induced in response to CCK in the course of pancreatic growth (14). We also detailed its role as a feedback inhibitor of CN-NFAT signaling and established the CN-NFAT-Rcan1 axis as the first molecular switch or negative feedback regulator of adaptive, hormonally-regulated pancreatic growth (14). Briefly, we showed that: (a) Rcan1 overexpression blocks CN-mediated nuclear translocation of NFAT in isolated acini; (b) Rcan1 overexpression both in isolated acini and in vivo blocks CN-mediated transcriptional activation of NFAT, as examined by NFAT-luciferase reporters; and (c) Rcan1 overexpression blocks NFAT-induced pro-proliferative gene expression driven by CCK. As the end-result of these changes, Rcan1 overexpression in vivo blocks CCK-driven acinar cell proliferation (assessed by BRDU incorporation) and adaptive growth of the pancreas. We also see that Rcan1

overexpression blocks CCK-induced activation of the Rcan1-luciferase reporter for the NFAT-dependent Rcan1.4 mRNA splice variant, thus forming an auto-inhibitory loop.

Furthermore, we examined the peak-to-trough kinetics of Rcan1 expression within the broader context of gene expression in early (0-8hr) pancreatic growth (15) and showed peak mRNA expression at 1-2hrs and a parallel increase in protein expression with a peak at 2-4 hours. We also briefly explored Rcan1expression along mid-long term (0-4days), observing a sinusoidal/pattern similar to that of other early response genes [Guo L and Gurda GT, unpublished work]. Though Rcan1 is known to interact with proteins and pathways other than CN and NFAT in other organs, those interactions in the pancreas remain largely unexplored. For further discussion on how CN-NFAT-Rcan1 fits within the broader context of pancreatic development, acinar cell maturation and function, please refer to a more detailed review (4).

The role of Rcan1 in the endocrine pancreas remains likewise poorly understood. Administration of 2-deoxyglucose has been shown to increase Rcan1 expression in islets of Langerhans, suggestive of its role in diabetes (6, 22). Studies to examine the role of Rcan1 specifically in β-cells are yet to be published, but parallel studies for CN-NFAT pathway (17) point to a potential role of Rcan1 within this part of the organ. In preliminary work, Rcan1 may affect insulin secretion [D.J. Keating group], as well as islet size and β-cell proliferation [Keating, DJ; Gurda GT and Williams JA – ongoing work].

4.Tools to Study Rcan1

(a) cDNA clones: Rcan1 cDNA clone with a GFP or a Myc tag is available from OriGene (Rockville, MD) and recombinant protein from Feldan Scientific, under DSCR1 (Baltimore, MD). Rcan1 targeted SiRNA from Dharmacon (Chicago, IL), had been successfully used in at least 3 papers cited in this review.

(b) Antibodies: Biocompare lists 84 different products by 12 companies. Our primary experience had been with a no longer available custom-made antibody. Comercially, we have had a generally positive experience with Aviva Biosystems polyclonal (Catalog# ARP38457_P050) and a mixed experience with a Sigma polyclonal antibodies (Western blot, IHC), Catalog# D6694 .

(c) Viral Vectors: Both Rcan1S and Rcan1L are available from Vector Biolabs (Philadelphia, PA). We had previously successfully used Rcan1 promoter-driven luciferase adenovirus (gift of Dr. Glembotski, San Diego State University) and Rcan1 adenovirus (gift of Dr. Beverly Rothermel, UTSW).

Mouse lines/phenotypes: Several strains of mice that harbor genetic modifications of Rcan1 have been characterized thus far. Inhibition of CN activity throughout the whole body has been shown to be embryonic lethal in mice (at E8), with defects in angiogenesis and heart valve formation. Among tissue-specific models, there is a cardiac-specific HA-tagged RCAN1 gene under a control of α-MHC promoter (32) and a “flox-on” conditional transgenic model that can be used with tissue-specific Cre-deleter mice (28). The most widely used model of RCAN1 deficient mice was generated using a targeted deletion of exons 4 and 5, which are necessary for CN binding (38). An alternative RCAN1 deficient mice, with a targeted deletion of exons 5 and 6, as well as similar RCAN2 deficient mice were also recently generated (35).

5. References

1. **Abbasi S, Lee JD, Su B, Chen X, Alcon JL, Yang J, Kellems RE, and Xia Y**. Protein kinase-mediated regulation of calcineurin through the phosphorylation of modulatory calcineurin-interacting protein 1. *J Biol Chem* 281: 7717-7726, 2006. PMID: 16415348

2. **Arron JR, Winslow MM, Polleri A, Chang CP, Wu H, Gao X, Neilson JR, Chen L, Heit JJ, Kim SK, Yamasaki N, Miyakawa T, Francke U, Graef IA, and Crabtree GR**. NFAT dysregulation by increased dosage of DSCR1 and DYRK1A on chromosome 21. *Nature* 441: 595-600, 2006. PMID: 16554754

3. **Baek KH, Zaslavsky A, Lynch RC, Britt C, Okada Y, Siarey RJ, Lensch MW, Park IH, Yoon SS, Minami T, Korenberg JR, Folkman J, Daley GQ, Aird WC, Galdzicki Z, and Ryeom S**. Down's syndrome suppression of tumour growth and the role of the calcineurin inhibitor DSCR1. *Nature* 459: 1126-1130, 2009. PMID:19458618

4. **Benitez CM, Goodyer WR, and Kim SK**. Deconstructing pancreas developmental biology. *Cold Spring Harb Perspect Biol* 4: 2012. PMID: 22587935

5. **Chan B, Greenan G, McKeon F, and Ellenberger T**. Identification of a peptide fragment of DSCR1 that competitively inhibits calcineurin activity in vitro and in vivo. *Proc Natl Acad Sci U S A* 102: 13075-13080, 2005. PMID: 16131541

6. **Crawford DR, Leahy KP, Abramova N, Lan L, Wang Y, and Davies KJ**. Hamster adapt78 mRNA is a Down syndrome critical region homologue that is inducible by oxidative stress. *Arch Biochem Biophys* 342: 6-12, 1997. PMID: 9185608

7. **Davies KJ, Ermak G, Rothermel BA, Pritchard M, Heitman J, Ahnn J, Henrique-Silva F, Crawford D, Canaider S, Strippoli P, Carinci P, Min KT, Fox DS, Cunningham KW, Bassel-Duby R, Olson EN, Zhang Z, Williams RS, Gerber HP, Perez-Riba M, Seo H, Cao X, Klee CB, Redondo JM, Maltais LJ, Bruford EA, Povey S, Molkentin JD, McKeon FD, Duh EJ, Crabtree GR, Cyert MS, de la Luna S, and Estivill X**. Renaming the DSCR1/Adapt78 gene family as RCAN: regulators of calcineurin. *Faseb J* 21: 3023-3028, 2007. PMID: 17595344

8. **Dawson TM, Steiner JP, Dawson VL, Dinerman JL, Uhl GR, and Snyder SH**. Immunosuppressant FK506 enhances phosphorylation of nitric oxide synthase and protects against glutamate neurotoxicity. *Proc Natl Acad Sci U S A* 90: 9808-9812, 1993. PMID: 7694293

9. **Ermak G, Morgan TE, and Davies KJ**. Chronic overexpression of the calcineurin inhibitory gene DSCR1 (Adapt78) is associated with Alzheimer's disease. *J Biol Chem* 276: 38787-38794, 2001. PMID: 11483593

10. **Espinosa AV, Shinohara M, Porchia LM, Chung YJ, McCarty S, Saji M, and Ringel MD**. Regulator of calcineurin 1 modulates cancer cell migration in vitro. *Clin Exp Metastasis* 26: 517-526, 2009. PMID: 19306109

11. **Fuentes JJ, Genesca L, Kingsbury TJ, Cunningham KW, Perez-Riba M, Estivill X, and de la Luna S**. DSCR1, overexpressed in Down syndrome, is an inhibitor of calcineurin-mediated signaling pathways. *Hum Mol Genet* 9: 1681-1690, 2000. PMID: 10861295

12. **Genesca L, Aubareda A, Fuentes JJ, Estivill X, De La Luna S, and Perez-Riba M**. Phosphorylation of calcipressin 1 increases its ability to inhibit calcineurin and decreases calcipressin half-life. *Biochem J* 374: 567-575, 2003. PMID: 12809556

13. **Gollogly LK, Ryeom SW, and Yoon SS**. Down syndrome candidate region 1-like 1 (DSCR1-L1) mimics the inhibitory effects of DSCR1 on calcineurin signaling in endothelial cells and inhibits angiogenesis. *J Surg Res* 142: 129-136, 2007. PMID:17610901

14. **Gurda GT, Crozier SJ, Ji B, Ernst SA, Logsdon CD, Rothermel BA, and Williams JA**. Regulator of Calcineurin 1 Controls Growth Plasticity of Adult Pancreas. *Gastroenterology* 139: 609-619, 2010. PMID: 20438729

15. **Gurda GT, Wang JY, Guo L, Ernst SA, and Williams JA**. Profiling CCK-mediated pancreatic growth: the dynamic genetic program and the role of STATs as potential regulators. *Physiol Genomics* 44: 14-24, 2012. PMID: 22010007

16. **Harris CD, Ermak G, and Davies KJ**. Multiple roles of the DSCR1 (Adapt78 or RCAN1) gene and its protein product calcipressin 1 (or RCAN1) in disease. *Cell Mol Life Sci* 62: 2477-2486, 2005. PMID: 16231093

17. **Heit JJ, Apelqvist AA, Gu X, Winslow MM, Neilson JR, Crabtree GR, and Kim SK**. Calcineurin/NFAT signalling regulates pancreatic beta-cell growth and function. *Nature* 443: 345-349, 2006. PMID: 16988714

18. **Hilioti Z, and Cunningham KW**. The RCN family of calcineurin regulators. *Biochem Biophys Res Commun* 311: 1089-1093, 2003. PMID: 14623294

19. **Hilioti Z, Gallagher DA, Low-Nam ST, Ramaswamy P, Gajer P, Kingsbury TJ, Birchwood CJ, Levchenko A, and Cunningham KW**. GSK-3 kinases enhance calcineurin signaling by phosphorylation of RCNs. *Genes Dev* 18: 35-47, 2004. PMID: 14701880

20. **Klee CB, Draetta GF, and Hubbard MJ**. Calcineurin. *Adv Enzymol Relat Areas Mol Biol* 61: 149-200, 1988. PMID: 2833077

21. **Kregel KC, and Zhang HJ**. An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. *Am J Physiol Regul Integr Comp Physiol* 292: R18-36, 2007. PMID: 16917020

22. **Leahy KP, Davies KJ, Dull M, Kort JJ, Lawrence KW, and Crawford DR**. adapt78, a stress-inducible mRNA, is related to the glucose-regulated protein family of genes. *Arch Biochem Biophys* 368: 67-74, 1999. PMID: 10415113

23. **Liu JO**. The yins of T cell activation. *Sci STKE* 2005. PMID: 15632417

24. **Minami T, Horiuchi K, Miura M, Abid MR, Takabe W, Noguchi N, Kohro T, Ge X, Aburatani H, Hamakubo T, Kodama T, and Aird WC**. Vascular endothelial growth factor- and thrombin-induced termination factor, Down syndrome critical region-1, attenuates endothelial cell proliferation and angiogenesis. *J Biol Chem* 279: 50537-50554, 2004. PMID: 15448146

25. **Minami T, Yano K, Miura M, Kobayashi M, Suehiro J, Reid PC, Hamakubo T, Ryeom S, Aird WC, and Kodama T**. The Down syndrome critical region gene 1 short variant promoters direct vascular bed-specific gene expression during inflammation in mice. *J Clin Invest* 119: 2257-2270, 2009. PMID: 19620774

26. **Mudd JO, and Kass DA**. Tackling heart failure in the twenty-first century. *Nature* 451: 919-928, 2008. PMID: 18288181

27. **Musaro A, McCullagh KJ, Naya FJ, Olson EN, and Rosenthal N**. IGF-1 induces skeletal myocyte hypertrophy through calcineurin in association with GATA-2 and NF-ATc1. *Nature* 400: 581-585, 1999. PMID: 10448862

28. **Oh M, Rybkin, II, Copeland V, Czubryt MP, Shelton JM, van Rooij E, Richardson JA, Hill JA, De Windt LJ, Bassel-Duby R, Olson EN, and Rothermel BA**. Calcineurin is necessary for the maintenance but not embryonic development of slow muscle fibers. *Mol Cell Biol* 25: 6629-6638, 2005. PMID: 16024798

29. **Pfister SC, Machado-Santelli GM, Han SW, and Henrique-Silva F**. Mutational analyses of the signals involved in the subcellular location of DSCR1. *BMC Cell Biol* 3: 24, 2002. PMID: 12225619

30. **Porta S, Serra SA, Huch M, Valverde MA, Llorens F, Estivill X, Arbones ML, and Marti E**. RCAN1 (DSCR1) increases neuronal susceptibility to oxidative stress: a potential pathogenic process in neurodegeneration. *Hum Mol Genet* 16: 1039-1050, 2007. PMID: 17341486

31. **Riper DV, Jayakumar L, Latchana N, Bhoiwala D, Mitchell AN, Valenti JW, and Crawford DR**. Regulation of vascular function by RCAN1 (ADAPT78). *Arch Biochem Biophys* 472: 43-50, 2008. PMID: 18294449

32. **Rothermel BA, McKinsey TA, Vega RB, Nicol RL, Mammen P, Yang J, Antos CL, Shelton JM, Bassel-Duby R, Olson EN, and Williams RS**. Myocyte-enriched calcineurin-interacting protein, MCIP1, inhibits cardiac hypertrophy in vivo. *Proc Natl Acad Sci U S A* 98: 3328-3333, 2001. PMID: 11248078

33. **Rothermel BA, Vega RB, and Williams RS**. The role of modulatory calcineurin-interacting proteins in calcineurin signaling. *Trends Cardiovasc Med* 13: 15-21, 2003. PMID: 12554096

34. **Rowan K**. Down syndrome offers fresh clues to angiogenesis. *J Natl Cancer Inst* 101: 1170-1171, 2009. PMID:19706624

35. **Sanna B, Brandt EB, Kaiser RA, Pfluger P, Witt SA, Kimball TR, van Rooij E, De Windt LJ, Rothenberg ME, Tschop MH, Benoit SC, and Molkentin JD**. Modulatory calcineurin-interacting proteins 1 and 2 function as calcineurin facilitators in vivo. *Proc Natl Acad Sci U S A* 103: 7327-7332, 2006. PMID: 16648267

36. **van Rooij E, Doevendans PA, Crijns HJ, Heeneman S, Lips DJ, van Bilsen M, Williams RS, Olson EN, Bassel-Duby R, Rothermel BA, and De Windt LJ**. MCIP1 overexpression suppresses left ventricular remodeling and sustains cardiac function after myocardial infarction. *Circ Res* 94: e18-26, 2004. PMID: 14739160

37. **Vega RB, Bassel-Duby R, and Olson EN**. Control of cardiac growth and function by calcineurin signaling. *J Biol Chem* 278: 36981-36984, 2003. PMID: 12881512

38. **Vega RB, Rothermel BA, Weinheimer CJ, Kovacs A, Naseem RH, Bassel-Duby R, Williams RS, and Olson EN**. Dual roles of modulatory calcineurin-interacting protein 1 in cardiac hypertrophy. *Proc Natl Acad Sci U S A* 100: 669-674, 2003. PMID:12515860

39. **Wang HG, Pathan N, Ethell IM, Krajewski S, Yamaguchi Y, Shibasaki F, McKeon F, Bobo T, Franke TF, and Reed JC**. Ca2+-induced apoptosis through calcineurin dephosphorylation of BAD. *Science* 284: 339-343, 1999. PMID: 10195903

40. **Yang J, Rothermel B, Vega RB, Frey N, McKinsey TA, Olson EN, Bassel-Duby R, and Williams RS**. Independent signals control expression of the calcineurin inhibitory proteins MCIP1 and MCIP2 in striated muscles. *Circ Res* 87: E61-68, 2000. PMID:11110780