we hope to see more articles and journals adopting this embedded-3D-structure format in the future and a concerted effort to improve the software needed so that it is accessible to and usable by everyone, non-specialist and specialist alike.

References

1 Kumar, P. *et al.* (2008) Grasping molecular structures through publication-integrated 3D models. *Trends Biochem. Sci.* 33, 408–412

2 Goodman, A.A. *et al.* (2009) A role for self-gravity at multiple length scales in the process of star formation. *Nature* 457, 63–66

3 Abagyan, R. *et al.* (2006) Disseminating structural genomics data to the public: from a data dump to an animated story. *Trends Biochem. Sci.* 31, 76–78

4 Hodis, E. *et al.* (2007) eMovie: a storyboard-based tool for making molecular movies. *Trends Biochem. Sci.* 32, 199–204

5 Porollo, A. and Meller, J. (2007) Versatile annotation and publication quality visualization of protein complexes using POLYVIEW-3D. *BMC Bioinformatics* 8, 316

6 Porollo, A.A. *et al.* (2004) POLYVIEW: a flexible visualization tool for structural and functional annotations of proteins. *Bioinformatics* 20, 2460–2462

7 Berman, H.M. *et al.* (2000) The Protein Data Bank. *Nucleic Acids Res.* 28, 235–242

8 Hodis, E. *et al.* (2008) Proteopedia - a scientific 'wiki' bridging the rift between three-dimensional structure and function of biomacromolecules. *Genome Biol.* 9, R121

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Research Focus

β-catenin gets jaded and von Hippel-Lindau is to blame

Jason D. Berndt, Randall T. Moon and Michael B. Major

Howard Hughes Medical Institute, Department of Pharmacology and Institute for Stem Cell and Regenerative Medicine, University of Washington School of Medicine, Box 357370, Seattle, WA 98109, USA

Numerous studies have pointed to interactions between the tumor suppressor von Hippel-Lindau (VHL) and the oncogenic Wnt–β-catenin signaling cascade; however, the mechanism of this crosstalk has remained elusive. Among other roles, VHL can promote the stabilization of Jade-1. Now, recent findings provide compelling evidence that Jade-1 ubiquitylates β-catenin, leading to its degradation. Thus, the loss of VHL, as seen in clear cell renal cell carcinoma, could lead to tumor formation through β-catenin de-repression.

Wnt–β-catenin signal transduction

Wnts comprise a conserved family of secreted glycoproteins. The founding member, *wingless* (*wg*), was initially characterized in *Drosophila melanogaster* and it was later found to be homologous to a murine proto-oncoprotein, INT-1 (Wg + INT = Wnt). Wnts are now known to be crucially important for numerous developmental processes and are required for regeneration in response to injury. Moreover, constitutive activation of the Wnt–β-catenin signal transduction pathway underlies the initiation and progression of several human cancers, most notably, colorectal carcinoma [1]. Recently, Chitalia *et al.* [2] described a new mechanism, via Jade-1 (gene for apoptosis and differentiation in epithelia), for communication between the Wnt–β-catenin and von Hippel-Lindau (VHL) tumor-suppressor pathways. Because VHL and Wnt–β-catenin signaling are implicated in related kidney pathologies, this finding underscores the clinical relevance of exploring this interaction.

β-catenin, a transcriptional co-activator, is required for canonical Wnt signal transduction. In the absence of Wnt ligand, β-catenin-dependent transcription is suppressed by multiple molecular mechanisms, including β-catenin ubiquitylation and proteasome-mediated degradation. A highly processive enzyme complex comprising casein kinase 1α, glycogen synthase kinase 3β (GSK3β), the adenomatosis polyposis coli protein (APC) and Axin phosphorylates conserved serine and threonine residues within the β-catenin N terminus. Phospho-β-catenin is then ubiquitylated by the Skp 1a-Cullin 1-β-transducin-repeat-containing protein ($SCF^{\beta TrCP}$) E3 ubiquitin ligase complex, thus targeting it for proteosomal degradation. In the presence of Wnt ligand, β-catenin phosphorylation and ubiquitylation is inhibited and, consequently, β-catenin levels increase. Similarly, genetic mutations that disrupt the phosphorylation or ubiquitylation complexes, such as those frequently observed within the *APC* gene in colorectal cancer, also stabilize β-catenin. As β-catenin accumulates, it translocates to the nucleus, binds members of the lymphoid enhancer-binding factor 1–T-cell specific transcription factor 7 (LEF–TCF) family of transcription factors, presumably displacing the co-repressors Groucho and C-terminal-binding protein. Transactivation of β-catenin target genes ensues, collectively controlling cellular differentiation, proliferation and migration [3].

Jade-1 negatively regulates β-catenin protein levels

Jade-1 contains two plant homeodomains (PHDs) and a PEST motif (named for its constituent amino acids, Pro-Glu-Ser-Thr) and is implicated as a tumor suppressor in renal cell carcinoma [4]. Chitalia *et al.* [2] identified β-catenin as one of nine interacting proteins in a yeast two-hybrid screen using a form of Jade-1 lacking the PHD domains as bait. Using endogenous, tagged and recombinant proteins, the authors convincingly demonstrate that Jade-1 directly binds β-catenin. Interestingly, Jade-1 binds the β-catenin N terminus, and this association is enhanced by

Corresponding author: Berndt, J.D. (jdberndt@u.washington.edu).

β-catenin serine–threonine phosphorylation. As expected, Jade-1–β-catenin binding is reduced in response to Wnt stimulation, which inhibits β-catenin phosphorylation.

Because Jade-1 binds the region of β-catenin required for its Wnt-dependent proteasomal degradation, the authors explored the hypothesis that Jade-1 negatively regulates β-catenin levels in the absence of Wnt stimulation. They show that endogenous β-catenin protein is reduced by 50–60% after Jade-1 overexpression. Likewise, Jade-1 silencing by short hairpin RNAs leads to a 100–150% increase in endogenous β-catenin protein levels. As expected from these binding data, overexpressed Jade-1 fails to efficiently degrade β-catenin in the presence of Wnt, in the absence of GSK3β activity, or when using a non-phosphorylatable form of β-catenin. Surprisingly however, the expression of a dominant-negative form of βTrCP does not interfere with Jade-1-mediated β-catenin destabilization, indicating that Jade-1 acts in parallel or downstream of βTrCP, the canonical β-catenin E3 ubiquitin ligase. Consistent with this observation, the authors provide compelling data that Jade-1 directly ubiquitylates β-catenin, both in cultured cells and *in vitro* using purified proteins [2]. Thus, Jade-1 seems to be the only E3 ligase, other than βTrCP, that regulates β-catenin ubiquitylation-degradation in response to canonical Wnt signaling.

The true test of any novel member of a signal transduction pathway is whether that protein can regulate signaling in whole organisms. Chitalia *et al.* [2] use the well-established Wnt-induced dorsalization assay in *Xenopus laevis* tadpoles to validate Jade-1 activity *in vivo*. Injecting *Xwnt8* or *β-catenin* mRNA into the ventral blastomeres of early blastulae causes axis duplication. However, co-injection of *Jade1* but not the PHD-deficient form, *Jade1-dd*, which cannot ubiquitylate β-catenin, partially rescues this phenotype. Thus, Jade-1 seems to be a novel, functionally conserved negative regulator of Wnt–β-catenin signaling.

VHL reduces β-catenin levels via Jade-1

VHL can positively regulate Jade-1 protein levels [5,6]. Thus, Chitalia *et al.* [2] tested the hypothesis that VHL and Jade-1 act in a linear pathway to reduce β-catenin protein levels. In a panel of renal cancer cell lines, β-catenin protein levels inversely correlated with deficiencies in *VHL*. Moreover, using VHL gain- and loss-of-function, they show that VHL negatively regulates β-catenin levels and the expression of two β-catenin target genes, *LEF1* and cyclin D1 (*CCND1*). To place Jade-1 between VHL and β-catenin in the pathway, the authors used a product of a *VHL* deletion mutant that cannot stabilize Jade-1. This form of VHL fails to degrade β-catenin in cultured mammalian cells and leads to increased expression of Wnt–β-catenin target genes in *Xenopus*. Likewise, Jade-1 knockdown by short hairpin RNA modestly inhibits the ability of overexpressed VHL to reduce β-catenin protein levels in cultured cells. In light of these data, more definitive experiments are needed to fully elucidate the epistatic relationship of VHL, Jade-1 and β-catenin. Nevertheless, the findings of Chitalia *et al.* [2] support a model whereby the loss of VHL activity, as seen in renal cancer, leads

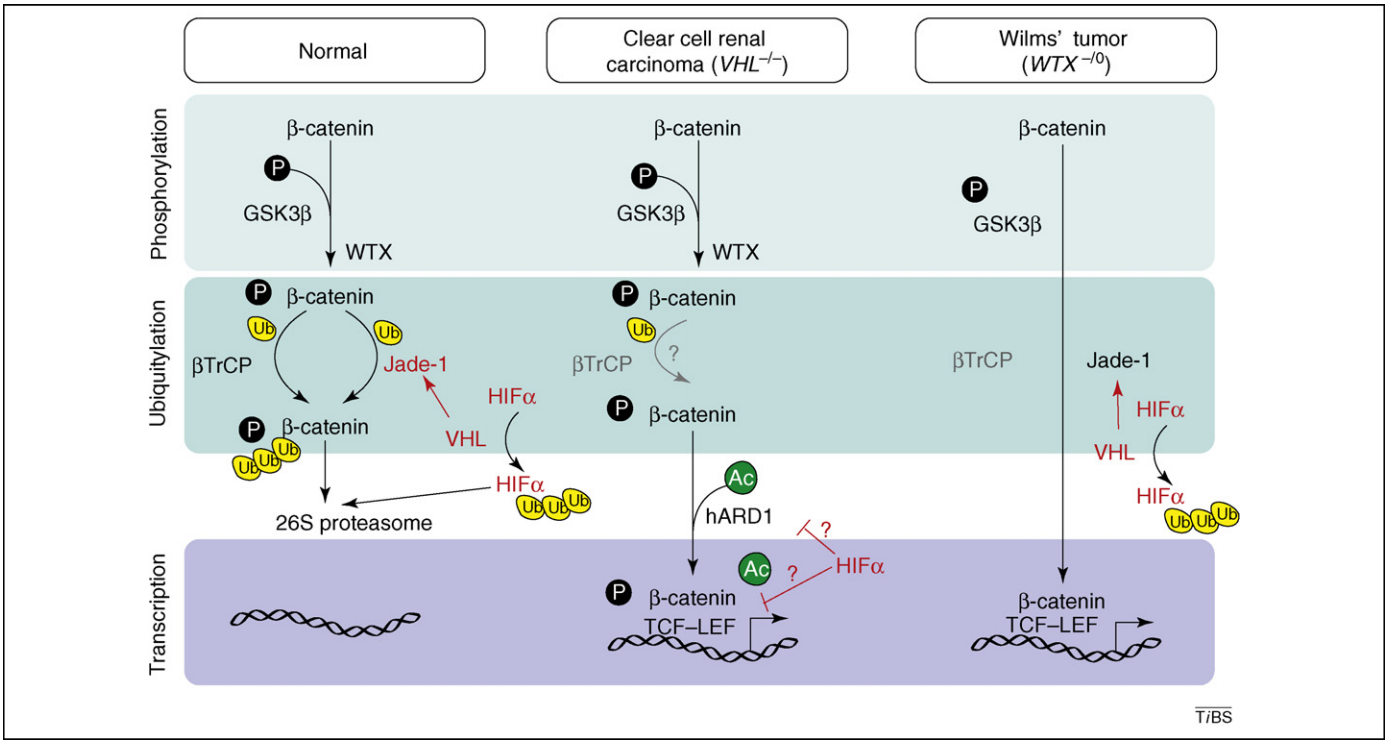

Figure 1. Intersecting signaling pathways contribute to renal cancer. The figure depicts a model for the interplay of VHL and β-catenin signaling in three cell types. In normal cells, VHL stabilizes the Jade-1 protein, which, in parallel with βTrCP, actively ubiquitylates β-catenin and leads to its proteasomal degradation (for clarity, the VHL–Jade-1–HIFα axis is shown in red). In *VHL* mutant clear cell renal carcinoma cells, the Jade-1 protein is unstable and, consequently, does not ubiquitylate β-catenin. Future studies are needed to address why βTrCP does not compensate for Jade-1 loss-of-function. Paradoxically, because VHL also regulates HIFα levels, it might simultaneously inhibit β-catenin activation by preventing its interaction with hARD1 and TCF–LEF. However, these mechanisms have yet to be tested in kidney cells. In Wilms' tumor, mutation of the *WTX* tumor suppressor gene prevents β-catenin phosphorylation and ubiquitylation by βTrCP and, presumably, by Jade-1. Because *VHL* mutations have not been reported in Wilms' tumor, HIFα levels remain low.

to reduced levels of Jade-1 and, consequently, to increased levels of β-catenin, thereby providing a novel molecular link between two cancer-relevant signal transduction pathways (Figure 1).

VHL regulates β-catenin through multiple mechanisms

VHL is best known as an E3 ubiquitin ligase that negatively regulates levels of the α subunit of hypoxia-inducible factor (HIF). HIF1 and HIF2 are heterodimeric transcription factors, whereas HIF3 lacks transcriptional activity and might negatively regulate HIF1 and HIF2. HIF-mediated transcriptional activity is required for the cellular adaptation to oxygen deprivation. Under normal physiologic conditions, proline residues within HIFα are hydroxylated, permitting constitutive VHL-mediated ubiquitylation and subsequent proteasomal degradation. By contrast, in a low oxygen environment such as that found within solid tumors, HIFα hydroxylation is reduced and, thus, stabilized. Similarly, mutations in *VHL* or hypermethylation of the *VHL* promoter lead to reduced VHL activity and thus promote HIFα stability [7].

HIF1α has been shown to negatively regulate β-catenin-dependent transcription. For example, HIF1α dissociates the acetyltransferase human arrest defective 1 (hARD1) from β-catenin, leading to decreased β-catenin acetylation and transcriptional activation in non-small-cell lung cancer cells [8]. Similarly, HIF1α directly competes with TCF4 for binding to β-catenin and thus inhibits β-catenin-mediated transcription in colorectal carcinoma cells [9]. It is unknown whether HIF2 or HIF3 can also affect β-catenin. HIF1α is selected against in renal carcinoma and, thus, it will be imperative to determine whether HIF2α can mimic the effects of HIF1α on β-catenin activity [7]. Nevertheless, it is likely that the VHL-mediated modulation of β-catenin-dependent transcription depends on the balance of its effects on HIFα and Jade-1 levels.

VHL might also affect Wnt–β-catenin signaling independently of HIF. Indeed, VHL inhibits β-catenin tyrosine phosphorylation, stabilization and nuclear translocation after hepatocyte growth-factor-stimulation in renal carcinoma cells [10]. Together, these studies combined with the recent Chitalia *et al.* [2] manuscript indicate that VHL can both positively and negatively regulate Wnt–β-catenin signaling in a variety of tissue types.

VHL–Jade-1–β-catenin signaling in kidney disease

VHL and Jade-1 are implicated as tumor suppressors in clear cell renal cell carcinoma (CCRCC). *VHL* mutations account for most familial and sporadic forms of CCRCC [7,11]; by contrast, Jade-1 overexpression in renal cancer cells decreases cell growth, colony formation and invasiveness in culture and slows tumor growth in mouse xenograft models [4]. Until now, Wnt–β-catenin signaling has not been linked to CCRCC, but it has an established role in another kidney cancer; mutations in *β-catenin* and in *FAM123B* (Wilms' tumor gene on X-chromosome, *WTX*), an $SCF^{\beta TrCP}$-associated negative regulator of β-catenin, are found in Wilms' tumor, a pediatric kidney cancer [12,13]. Thus, future studies designed to define the *in vivo* contributions of the VHL–Jade-1 and Wnt–β-catenin pathways to these and related cancers are of immediate concern.

In addition to tumors, patients with VHL disease often have polycystic kidneys. VHL localizes to cilia and is thought to have a role in orienting microtubules; it also is required for ciliogenesis [14]. Intriguingly, there are several connections between Wnt–β-catenin signaling and cilia. For example, *PKD1*, the gene most frequently disrupted in autosomal-dominant polycystic kidney disease, is a transcriptional target of β-catenin–TCF [15]. Moreover, the *PKD1* protein, Polycystin-1, forms a complex with β-catenin and E-cadherin at the membrane [16]. The knockdown or genetic loss of various ciliary proteins in a variety of systems promotes exaggerated Wnt signaling [17]. Importantly, the loss of *APC* in mouse kidneys leads to elevated β-catenin levels and highly cystic kidneys, providing substantial evidence for the role of Wnt–β-catenin signaling in kidney pathology [18]. Thus, communication between VHL and β-catenin could be relevant to the formation of kidney cysts. However, a direct link between VHL in cilia and Wnt–β-catenin inhibition has yet to be established. Likewise, nothing is known about a role for Jade-1 in cilia.

Unresolved issues

In conclusion, the discovery that VHL–Jade-1 regulates β-catenin elicits many more questions than it answers. For example, what is the relationship between Jade-1 and other components of the canonical Wnt signaling pathway? Does Jade-1 interact with other members of the β-catenin destruction complex or with WTX? Similarly, Jade-1 is one of at least five reported E3 ligases that can ubiquitylate β-catenin. Although Chitalia *et al.* [2] begin to address the relationship between Jade-1 and $SCF^{\beta TrCP}$, more rigorous experiments are required and the role(s) of other E3 ubiquitin ligases must be addressed. If these E3 ligases in fact act in isolation, what is the context in which each is recruited? In addition, Jade-1 is reported to promote histone acetyltransferase (HAT) activity [19,20]. Does Jade-1-dependent HAT activity affect β-catenin levels or β-catenin-mediated transcription? Finally, a connection probably exists between the requirement of VHL in ciliogenesis and cilia-mediated inhibition of Wnt–β-catenin signaling. Does the effect of VHL on β-catenin rely on its ciliary localization or function? Perhaps more important than the insight provided from future mechanistic studies will be determining whether mutational inactivation of *VHL* correlates with the expression of β-catenin target genes in human kidney cancer and cystic kidney disease.

References

1 Moon, R.T. *et al.* (2004) WNT and β-catenin signaling: diseases and therapies. *Nat. Rev. Genet.* 5, 691–701
2 Chitalia, V.C. *et al.* (2008) Jade-1 inhibits Wnt signalling by ubiquitylating β-catenin and mediates Wnt pathway inhibition by pVHL. *Nat. Cell Biol.* 10, 1208–1216
3 Kimelman, D. and Xu, W. (2006) β-catenin destruction complex: insights and questions from a structural perspective. *Oncogene* 25, 7482–7491
4 Zhou, M.I. *et al.* (2005) Jade-1, a candidate renal tumor suppressor that promotes apoptosis. *Proc. Natl. Acad. Sci. U. S. A.* 102, 11035–11040
5 Zhou, M.I. *et al.* (2004) Tumor suppressor von Hippel-Lindau (VHL) stabilization of Jade-1 protein occurs through plant homeodomains and is VHL mutation dependent. *Cancer Res.* 64, 1278–1286
6 Zhou, M.I. *et al.* (2002) The von Hippel-Lindau tumor suppressor stabilizes novel plant homeodomain protein Jade-1. *J. Biol. Chem.* 277, 39887–39898

7 Kaelin, W.G., Jr (2008) The von Hippel-Lindau tumour suppressor protein: O_2 sensing and cancer. *Nat. Rev. Cancer* 8, 865–873
8 Lim, J.H. *et al.* (2008) Hypoxia-inducible factor-1α obstructs a Wnt signaling pathway by inhibiting the hARD1-mediated activation of β-catenin. *Cancer Res.* 68, 5177–5184
9 Kaidi, A. *et al.* (2007) Interaction between β-catenin and HIF-1 promotes cellular adaptation to hypoxia. *Nat. Cell Biol.* 9, 210–217
10 Peruzzi, B. *et al.* (2006) The von Hippel-Lindau tumor suppressor gene product represses oncogenic β-catenin signaling in renal carcinoma cells. *Proc. Natl. Acad. Sci. U. S. A.* 103, 14531–14536
11 Lonser, R.R. *et al.* (2003) von Hippel-Lindau disease. *Lancet* 361, 2059–2067
12 Major, M.B. *et al.* (2007) Wilms tumor suppressor WTX negatively regulates WNT/β-catenin signaling. *Science* 316, 1043–1046
13 Rivera, M.N. *et al.* (2007) An X chromosome gene, WTX, is commonly inactivated in Wilms tumor. *Science* 315, 642–645
14 Kuehn, E.W. *et al.* (2007) Von Hippel-Lindau: a tumor suppressor links microtubules to ciliogenesis and cancer development. *Cancer Res.* 67, 4537–4540
15 Rodova, M. *et al.* (2002) The polycystic kidney disease-1 promoter is a target of the β-catenin/T-cell factor pathway. *J. Biol. Chem.* 277, 29577–29583
16 Huan, Y. and van Adelsberg, J. (1999) Polycystin-1, the PKD1 gene product, is in a complex containing E-cadherin and the catenins. *J. Clin. Invest.* 104, 1459–1468
17 He, X. (2008) Cilia put a brake on Wnt signalling. *Nat. Cell Biol.* 10, 11–13
18 Qian, C.N. *et al.* (2005) Cystic renal neoplasia following conditional inactivation of apc in mouse renal tubular epithelium. *J. Biol. Chem.* 280, 3938–3945
19 Foy, R.L. *et al.* (2008) Role of Jade-1 in the hat HBO1 complex. *J. Biol. Chem.* 283, 28817–28826
20 Panchenko, M.V. *et al.* (2004) von Hippel-Lindau partner Jade-1 is a transcriptional co-activator associated with histone acetyltransferase activity. *J. Biol. Chem.* 279, 56032–56041

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Research Focus

A caspase homolog keeps CED-3 in check

Graham F. Brady[1] and Colin S. Duckett[1,2]

[1] Department of Pathology, University of Michigan Medical School, M5240 Medical Science I, 1301 Catherine Street, Ann Arbor, MI 48109, USA
[2] Department of Internal Medicine, University of Michigan Medical School, 3110 Taubman Center, SPC 5368, 1500 East Medical Center Drive, Ann Arbor, MI 48109, USA

Apoptosis is a highly conserved form of cell death that is essential for controlling cell numbers throughout the lifetime of an organism. In *Caenorhabditis elegans*, the final step in the apoptotic cascade is activation of the death-inducing protease CED-3. Until now, no direct negative regulators of CED-3 had been identified, so the mechanism for maintaining a proper life–death balance was unclear. Now, a new study identifies CSP-3 as an important negative regulator of CED-3 during *C. elegans* development.

Apoptosis: a conserved and highly regulated cell death program

The apoptotic cell death program is highly conserved from invertebrates to mammals and is mediated by a set of death-inducing proteases called caspases (cysteine-dependent aspartate-specific proteases), which cleave cellular substrates in a highly specific and regulated manner [1,2]. The importance of apoptosis in the development of multicellular organisms has been described particularly well for the nematode *Caenorhabditis elegans* (for a review, see Ref. [3]). Throughout *C. elegans* development, cell numbers are precisely controlled in the developing organs and virtually all adult worms contain precisely the same number of cells in each mature organ. Several mechanisms are in place to regulate this process, ensuring that the *C. elegans* executioner caspase CED-3 is triggered to execute apoptosis in cells that should die but is not mistakenly activated in cells that should survive. Now, a recent study by Geng *et al.* [4] provides a novel mechanism for keeping CED-3 inactive in cells that should not undergo apoptosis.

Apoptosis in *C. elegans* is broadly similar to apoptosis in higher organisms (Table 1) and CED-3 possesses substantial homology to mammalian caspase-3 and caspase-8 [5–7]. Like other caspases, CED-3 is synthesized as an inactive zymogen; dimerization and autoproteolysis generate the active components (the large and small subunits) from the N-terminal prodomain [8]. Inactive CED-3 monomers are brought together and activated by oligomerized CED-4, a process that is analogous to mammalian caspase-9 activation [9,10]. In species from flies to humans, caspase activation and proteolytic activity are subject to negative regulation by the inhibitor of apoptosis (IAP) protein family [11,12]. However, the two IAPs encoded in the *C. elegans* genome are thought to participate in cytokinesis, not apoptosis [13]. Given the similarities between *C. elegans* and mammalian apoptosis, the apparent absence of IAPs or other caspase inhibitors to keep CED-3 in check is puzzling. By contrast, in *Drosophila melanogaster*, the activity of IAPs, specifically DIAP1 (for *Drosophila* IAP1), is crucial in preventing uncontrolled caspase activity and apoptosis [14]. In mammals, another level of caspase regulation is provided by a group of caspase-like decoy proteins [15]. Some of these proteins contain only a caspase-recruitment domain (CARD), whereas others resemble full-length caspases but lack the crucial catalytic cysteine residue. These decoy molecules can exert their anti-apoptotic effects by binding and sequestering procaspase zymogens or by competing with caspases for insertion into caspase-activating complexes. Until now, no caspase-like decoy proteins had been identified in *C.*

Corresponding author: Duckett, C.S. (colind@umich.edu).