

## Partners in crime

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**Caspases have apoptotic as well as non-apoptotic functions, both of which depend on their abilities to cleave proteins at specific sites. What distinguishes apoptotic from non-apoptotic substrates has so far been unclear. In this issue, Weaver et al. (2017) now provide an answer to this crucial question.**

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Caspases are cysteine-dependent aspartate-specific proteases that are well known for their essential, conserved roles in apoptotic cell death. However, in recent years, it has become clear that caspases are involved in processes other than apoptosis. Indeed, the founding member of the caspase family, *C. elegans* CED-3, has now been implicated in processes ranging from neuronal regeneration to aging (Nakajima and Kuranaga, 2017). The *ced-3* gene was originally identified in a genetic screen for mutants in which many of the 131 somatic cells that reproducibly die during *C. elegans* development fail to do so (Ellis and Horvitz, 1986). The subsequent cloning of

the *ced-3* gene led to the discovery that the gene encodes a protease, and this was the first step towards the elucidation of the molecular mechanisms underlying apoptotic cell death (Conradt et al., 2016). The current model for how *ced-3* promotes apoptosis in *C. elegans* is that in cells ‘programmed’ to die, the pro-form of CED-3 (i.e. proCED-3) is converted into the mature, fully active CED-3 enzyme, which is composed of two small (p15) and two large (p17) subunits. CED-3 activation in this context is triggered by the BH3-only protein EGL-1 and mediated by the apoptosome, which is composed of eight subunits of the Apaf1-like protein CED-4. CED-3 then cleaves various protein substrates at specific sites (C-terminal to the amino acid sequence DxxD), thereby either inactivating their anti-apoptotic function or activating their apoptotic function, and thus triggering the cellular processes necessary for the controlled dismantling and engulfment of the cell. For example, the cleavage and activation by CED-3 of the putative transporter CED-8 Xkr is required for the exposure on the cell surface of phosphatidylserine, which is critical for the recognition of the apoptotic cell by phagocytes (Chen et al., 2013; Suzuki et al., 2013).

A few years ago, Min Han and colleagues ‘rediscovered’ the *ced-3* gene in a screen for mutations that enhance the developmental defects caused by compromised microRNA-induced silencing complex (miRISC) function (Weaver et al., 2014). By reducing the stability and/or translation of mRNAs, miRISC represses the expression of numerous genes, including the heterochronic gene *lin-28* (Ambros, 2011). *lin-28* encodes an RNA-binding protein that is necessary for the timely occurrence of processes that take place specifically during the second larval (L2) stage of *C. elegans* development (Moss et al., 1997). For example, in wild-type animals the epidermal seam cells (which are stem cell-like) undergo a specific pattern of cell divisions during the L2 stage. Mutations that inactivate *lin-28* result in the ‘skipping’ of these

cell divisions, and thus a reduction in the number of seam cells in adult animals (the *lin-28* loss-of-function [lf] phenotype). In wild-type animals, miRISC-mediated repression of *lin-28* expression occurs towards the end of the L2 stage, and this is critical for the transition to the L3 stage and the timely occurrence of L3-specific processes. Inappropriate elevation of LIN-28 protein levels in the seam cells results in the ‘reiteration’ of the L2-specific cell division patterns and an increase in the number of seam cells in adults (the *lin-28* gain-of-function [gf] phenotype). In their 2014 publication, Han and colleagues demonstrated that the inactivation of *ced-3* function in a genetic background where miRISC function is compromised greatly enhances the *lin-28* gf phenotype (Weaver et al., 2014). Furthermore, they showed that LIN-28 protein is a substrate of the CED-3 caspase *in vitro* and that CED-3 proteolytically cleaves LIN-28 at a specific site (28DVVD31) to generate a truncated LIN-28 protein that lacks the first 31 amino acids. Finally, they presented evidence that *ced-3* function is necessary for the efficient loss of LIN-28 protein at the L2-L3 transition. Based on these findings, the authors proposed that miRISC and CED-3 function redundantly in the repression of *lin-28* expression and thereby contribute to the robustness of developmental transitions during *C. elegans* development (Weaver et al., 2014). In the current issue of *Development Cell*, Weaver and colleagues (2017) now go one step further in their analysis of this non-apoptotic function of the CED-3 caspase . Their findings uncover a mechanism that may represent a general paradigm for the recognition and inactivation of non-apoptotic caspase substrates.

The impetus for this most recent report stemmed from two observations that suggested CED-3 caspase might cooperate with another protein degradation system to reduce the level of LIN-28 protein at the L2-L3 transition. First, the truncated LIN-28 protein generated by *ced-3*-dependent

cleavage can rescue the *lin-28* lf phenotype, and therefore is functional *in vivo*. For this reason, there must be an additional mechanism that leads to reduced LIN-28 activity. Second, the truncated LIN-28 protein contains an N-terminal asparagine and, hence, could potentially be subject to degradation by the Arg/N-end rule pathway (Varshavsky, 2011). Indeed, Weaver and colleagues (2017) now provide *in vitro* and *in vivo* evidence that *ced-3*-dependent cleavage of LIN-28 generates an Arg/N-end rule degron. In addition, they demonstrate that, like the loss of *ced-3*, the loss of either *ubr-1* or *ate-1* greatly enhances the *lin-28* gf phenotype of animals in which miRISC function is compromised (*ubr-1* encodes a UBR-type E3 ubiquitin ligase, and *ate-1* an arginyltransferase, both of which are required for protein degradation mediated by the Arg/N-end rule pathway). Furthermore, they provide genetic evidence that in the context of the control of *lin-28* expression, the activities of CED-3 caspase and the Arg/N-end rule pathway are mutually dependent. This notion is further supported by the demonstration that the UBR-1 and ATE-1 proteins physically interact with CED-3 caspase. Based on these findings, Weaver and colleagues put forward the model that a protein complex comprising CED-3, UBR-1 and ATE-1 binds to LIN-28, and this results in *ced-3*-dependent cleavage of LIN-28 protein at 28DVVD<sub>31</sub>. The truncated LIN-28 protein is then subject to *ubr-1*- and *ate-1*-dependent degradation through the Arg/N-end rule pathway (Weaver et al., 2017).

Are these observations representative of a general phenomenon in *C. elegans*? It remains to be determined whether other non-apoptotic CED-3 substrates are also recognized by the CED-3, UBR-1, ATE-1 complex instead of being bound by CED-3 alone (as is presumably the case for apoptotic CED-3 substrates). And is ‘partnering up’ with components of the Arg/N-end rule pathway also utilized by caspases in mammalian cells to recognize non-apoptotic substrates and control their levels? This could well be the case, since Weaver and colleagues (2017) report that

several mammalian caspases (including Caspases 1, 3 and 8) interact with human UBR2 protein *in vitro*. Other exciting and important questions also remain to be addressed in the context of *C. elegans* development, and in the context of the control of *lin-28* expression. In particular, how is the maturation and activation of CED-3 caspase regulated in non-apoptotic cells? And, how is the activity of the CED-3, UBR-1, ATE-1 complex controlled? Finally, it is worth noting that both the apoptotic function of *ced-3* and the anti-apoptotic function of *ced-3* in the control of developmental transitions were discovered through forward genetic screens. This serves as a reminder of the timeless power of unbiased experimental approaches and of genetic models such as *C. elegans*.

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