Programmed cell death or apoptosis requires activation of cysteinyl aspartate proteases called caspases. These proteases are present in an inactive form in all metazoan cells and function in a cascade whereby initiator caspases cleave and activate executioner caspases. Despite the importance of these enzymes for cell death, the activation of initiator caspases is still not fully understood. The activation of the apical protease, caspase-8, is thought to occur as a result of its ligand-induced recruitment to a receptor of the TNF superfamily, such as DR4. Receptor stimulation drives the formation of caspase-8 dimers, which then undergo auto-activation (Boatright et al., 2003; Donepudi et al., 2003). Full activation of caspase-8, however, requires higher order aggregation. Jin et al. (2009) now demonstrate that ubiquitylation of caspase-8 and its subsequent translocation to cytosolic aggregates or speckles contribute to the full activation of caspase-8 and subsequent cell death upon TRAIL ligand signaling.

Ubiquitylation reactions at signaling receptors can drive many different outcomes, including activation and trafficking. Jin et al. while looking at such modifications at DR4 noticed that caspase-8 became ubiquitylated and, using size-exclusion chromatography, found the highest caspase-8 cleavage activity in the high molecular weight fractions. To identify which E3 ligase might perform the caspase modification, the authors used tandem mass spectrometry to analyze the active fraction and discovered Cullin3 (CUL3). Knockdown of CUL3 abolished ubiquitylation of caspase-8 and reduced TRAIL-induced activation of caspase-8. Conversely, overexpression of CUL3 increased caspase-8 ubiquitylation and activation.

In vitro assays showed that CUL3 was able to ubiquitylate the C-terminal region of caspase-8 with both K48- and K63-linked polyubiquitin (pUb) chains. The addition of a proteasomal inhibitor did not affect levels of caspase-8, suggesting that the K48 and K63 pUb chains in this case did not signal for degradation of caspase-8. This is rather surprising because K48 pUb chains typically promote proteasomal degradation, whereas K63 pUb chains are associated with a variety of consequences, such as signaling. Whether K48 and K63 pUb chains perform the same function in this situation remains to be determined. Jin et al., however, are not the first to show that ubiquitylation of caspases alters their activity. Ditzel et al. (2008) recently showed that drICE, the homolog of mammalian caspase-3, was polyubiquitylated by DIAP1, with K48 and K63 pUb chains. These K48 pUb chains also did not lead to degradation, but in contrast to the results from Jin et al., ubiquitylation inhibited rather than increased drICE activity.

CUL3 was found in the DISC complex isolated using a DR4-specific antibody. One potential mechanism for recruitment of CUL3 to the membrane is through DCNL1-like protein 3 (DCNL3). Unlike the other members of the DCNL family, this molecule is located at the membrane through myristoylation and palmitoylation modifications. These modifications of DCNL3 are required for neddylation of CUL3, a modification which controls the activity of CUL3 to function as an E3 ligase (Meyer-Schaller et al., 2009). Therefore, CUL3 may already be present and active at the membrane prior to activation of DR4.

Cullins normally function as scaffolds that bring together a RING protein RBX1 and a substrate binding protein containing a Bric-a-brac-Tramtrack-Broad (BTB) domain. Although RBX1 was required for caspase-8 ubiquitylation, RBX1 was not found in the DISC complex by either immunoprecipitation or mass spectrometry approaches and likewise no BTB-containing protein was detected. This leaves open the question whether the CUL3 complex architecture in the DISC is very different to previously described CUL3 complexes or whether these proteins were not detected due to a technical issue. Cullins have recently been implicated in mitosis, and CUL3, in particular, has been shown to modify Aurora B, a protein involved in M phase progression (Sumara et al., 2008). It is tempting to speculate that the interaction of CUL3 and caspase-8 suggests a mechanism for the reported alternative roles of caspase-8 such as that in T cell expansion (Bell et al., 2008).

If caspase-8 can be ubiquitylated in a CUL3-dependent manner to increase activity, then deubiquitylation of caspase-8 may also provide a mechanism to decrease activity. In line with this idea, A20 was found to be able to deubiquitylate caspase-8 and thereby limit aggregation and subsequent activation of caspase-8. This result suggests that there is yet

Ubiquitylation of caspase-8 by the Cullin3 E3 ligase allows its translocation to cytosolic aggregates in the cell by p62/sequestosome-1, increasing caspase-8 activation and thus leading to TRAIL-induced cell death.
another point of regulation for caspase-8 activity. However, Jin et al. did not examine whether other deubiquitylating enzymes can also perform this function and what physiological circumstances may cause this action to occur.

In addition to finding CUL3, p62/sequestosome-1 was also identified in the DISC complex but only after caspase-8 was ubiquitylated by CUL3. Knockdown of p62 showed that p62 was required for aggregation and relocation of ubiquitylated caspase-8 to Ub-rich aggregates or speckles within the cell resulting in increased caspase-8 activity and subsequent TRAIL-induced cell death. This suggests that p62 acts downstream of CUL3. Interestingly, knockdown of either CUL3 or p62 reduced caspase-8 activity but never abolished activity completely. This either is due to incomplete knockdown of either molecule or supports the model that ligand stimulation does indeed activate a limited number of caspase-8 molecules.

In addition to the role that p62 plays in the full activation of caspase-8, it is unclear what role p62 plays in TRAIL-induced activation of NF-κB. Genetic deletion of p62 leads to inhibition of IKK activation and NF-κB translocation in RANK ligand-stimulated osteoclastogenesis due to loss of interaction with TRAF6 and αPKC (Durán et al., 2004). Therefore, it is likely that additional proteins besides p62 and ubiquitylated caspase-8 are present at these cytoplasmic speckles. What signaling molecules and how they are recruited to p62 and caspase-8 may indicate further downstream signaling events important to the outcome of the cell’s survival.

Previous work from this group showed that caspase-8 was required for activation of MAPK pathways and NF-κB by TRAIL (Varfolomeev et al., 2005). Furthermore, their work showed that activation of caspase-8 was required for the subsequent signaling through JNK and p38 while generating sustained NF-κB signaling (Varfolomeev et al., 2005). Taken together with their new work, one question that arises is whether the increased level of caspase-8 activity limits or enhances this signaling.

Ubiquitylation of caspase-8 was also found upon stimulation with other pro-apoptotic TNF superfamily ligands, suggesting a global mechanism of caspase-8 activation. This is an intriguing idea; however, there are key differences between TRAIL and TNFα signaling. For example, the caspase-containing DISC complex does not form at the receptor upon TNFα stimulation but in the cytoplasm and TNFα signaling does not normally cause cell death because activation of anti-apoptotic genes blocks DISC activity from TNFα signaling. Therefore, it is an open question whether ubiquitylation of caspase-8 by CUL3 occurs in TNFα signaling, and if so what role it might play there.

Although p62 knockout mice did not present with a phenotype similar to other death receptor knockouts such as Fas knockout, a role for p62 was found upon stimulation of the RANK-L pathway, suggestive of its involvement in osteoclastogenesis (Durán et al., 2004). Clearly, closer inspection of p62 knockout mice is warranted because TRAIL and TNFα knockout mice are phenotypically normal until challenged.

The work performed by Jin et al. reveals CUL3 and p62 as critical players in the activation of caspase-8 for TRAIL-induced cell death. Other recent findings demonstrate that viruses can hijack cululin function to promote or inhibit degradation of specific host proteins allowing viral replication (Barry and Früh, 2006), highlighting the importance of CUL3 in disease. Increased levels of p62 promote tumorigenesis either in combination with activated Ras or as a result from impaired autophagy (Durán et al., 2008; Mathew et al., 2009). On the basis of the results from Jin et al., mutation or disruption of the function of CUL3 or p62 is now also a potential mechanism of resistance to TRAIL-based therapies, and it will be interesting to see whether a correlation does exist that will speed up the use of these promising reagents in the clinic.

References